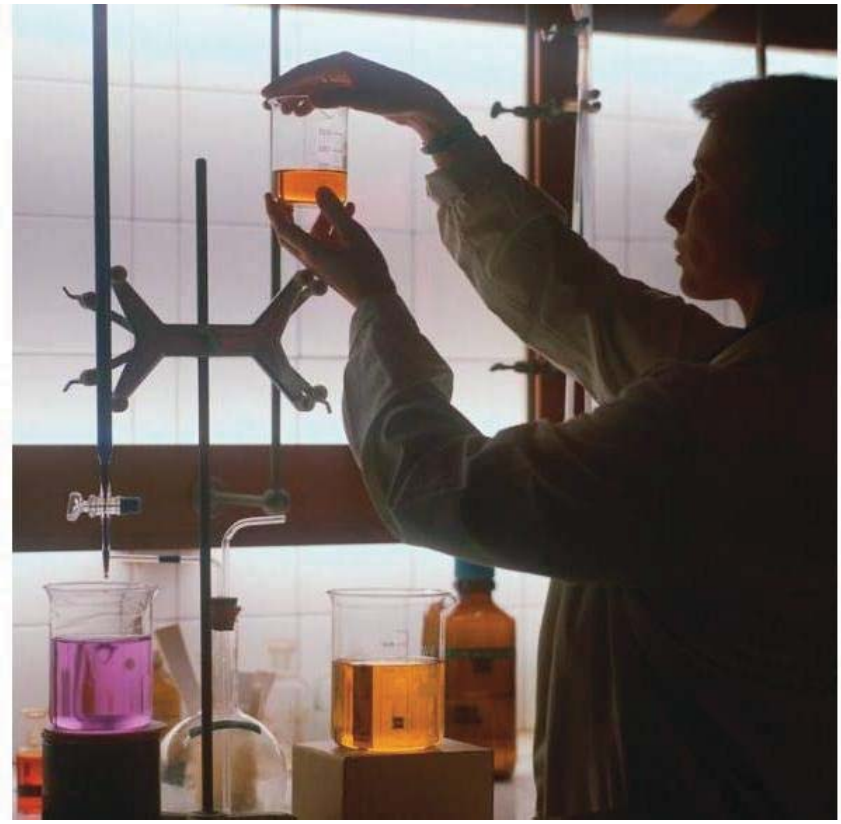
Wisconsin Department of Natural Resources
Wastewater Operator Certification

Advanced On-site Laboratory Testing Study Guide

August 2010 Edition (Revised January 2013)



Subclass J

Wisconsin Department of Natural Resources
Bureau of Science Services
Operator Certification Program
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Preface

This operator's study guide represents the results of an ambitious program. Operators of wastewater facilities, regulators, educators and local officials, jointly prepared the objectives and exam questions for this subclass.

January 2013 Revisions: Minor revisions were made to the January 2010 edition of this study guide. The following key knowledges have been updated: 2.2.1, 3.1.3, 3.2.2, 3.2.3, 3.2.6, 5.2.7, 5.4.3, 5.4.7, 5.7.4, 5.7.5, 6.2.7

How to use this study guide with references.

In preparation for the exams you should:

1. Read all of the key knowledge's for each objective.

2. Use the resources listed at the end of the study guide for additional information.

3. Review all key knowledge's until you fully understand them and know them by memory.

It is advisable that the operator take classroom or online training in this process before attempting the certification exam.

Choosing A Test Date

Before you choose a test date, consider the training opportunities available in your area. A listing of training opportunities and exam dates is available on the internet at http://dnr.wi.gov, keyword search "operator certification". It can also be found in the annual DNR "Certified Operator" or by contacting your DNR regional operator certification coordinator.

Acknowledgements

This Study Guide is a result of the combined efforts of the following individuals:

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Chapter 1 - Safety

Section 1.1 - Definitions

1.1.1 Abbreviations used in this document

Figure 1.1.1.1

ACS	American Chemical Society
AR Grade	Analytical Reagent Grade
ASTM	American Society for Testing and Materials
BOD	Biochemical Oxygen Demand
°C	Degrees Celsius (temperature)
cBOD	carbonaceous Biochemical Oxygen Demand
CCV	Continuing Calibration Verification
CFR	Code of Federal Regulations
COC	Chain-of-Custody
COD	Chemical Oxygen Demand
DMR	Discharge Monitoring Report
DNR	Department of Natural Resources
DO	Dissolved Oxygen
EP	Extraction Procedure
EPA	Environmental Protection Agency
GGA	Glucose - Glutamic Acid solution (for BOD)
HEM	Hexane Extractable Materials
ICV	Initial Calibration Verification
ID	Identification
IDC	Initial Demonstration of Capability
ISE	Ion Selective Electrode
LCS	Laboratory Control Sample
LOD	Limit of Detection
LOQ	Limit of Quantitation
MDL	Method Detection Limit
mL	milliliter
MLSS	Mixed Liquor Suspended Solids
MS	Matrix Spike
MSD	Matrix Spike Duplicate
MSDS	Material Safety Data Sheet
mV	millivolts

NFR	Non-Filterable Residue (See TSS)
NIST	National Institute of Standards and Technology
NPDES	National Pollutant Discharge Elimination System
NR 149	Laboratory Certification and Registration Code; Wisconsin Administrative Code
ppb	part per billion
ppm	part per million
PT	Proficiency Testing sample (formerly called reference samples)
QA	Quality Assurance
QC	Quality Control
QCS	A reference standard obtained externally that comes with acceptance criteria. Formerly known as "blind samples".
R	Correlation coefficient, seen as lower case, " r "
RPD	Relative Percent Difference
RSD	Relative Standard Deviation
SOP	Standard Operating Procedure
SRM	Standard Reference Material
SVI	Sludge Volume Index
TC	To Contain
TDS	To Deliver
TDS	Total Dissolved Solids
TRC	Total Residual Chlorine
TSS	Total Suspended Solids
UV	Ultraviolet (a means of disinfection)
WPDES	Wisconsin Pollution Discharge Elimination System
WWTP	Wastewater Treatment Plant

1.1.2 Define MSDS.

MSDS= Material Safety Data Sheet

A material safety data sheet (MSDS) is a form containing data regarding the properties of a particular substance. An important component of workplace safety, MSDS sheets are

intended to provide workers and emergency personnel with procedures for handling or working with that substance in a safe manner. Critical information contained in MSDS sheets include physical data (melting point, boiling point, flash point, etc.), toxicity, health effects, first aid, reactivity, storage, disposal, protective equipment, and spill handling procedures. The exact format of an MSDS can vary from source to source within a country depending on specificity of the national requirement.

The 16-section MSDS is becoming the international norm. The 16 sections are:

* Identification
* Hazard(s) identification
* Composition/information on ingredients
* First-aid measures
* Fire-fighting measures
* Accidental release measures
* Handling and storage
* Exposure controls/personal protection
* Physical and chemical properties
* Stability and reactivity
* Toxicological information
* Ecological information
* Disposal considerations
* Transport information
* Regulatory information
* Other information

An MSDS should be available for all chemicals you use. If a chemical doesn't come with an MSDS, one should be obtained online. Keep a file for all MSDS sheets.

1.1.3 Define Chemical Hygiene Plan.

Chemical Hygiene Plans (CHP) are required by federal regulation under 29 CFR Part 1910. This code is part of what is often referred to as the “Employees Right to Know” Act.

Title 29— Labor
PART 1910—OCCUPATIONAL SAFETY AND HEALTH STANDARDS
Subpart Z—Toxic and Hazardous Substances

Chemical Hygiene Plan means a written program developed and implemented by the employer which establishes procedures, safety equipment, personal protective equipment, and work practices that are capable of protecting employees from the health hazards presented by hazardous chemicals used in that particular workplace and must meet specific requirements summarized below. The employer MUST review and evaluate the effectiveness of the Chemical Hygiene Plan at least annually and update it as necessary.

Where hazardous chemicals (as defined by 29 CFR Part 1910) are used in the workplace, the employer must develop and maintain the provisions of a written Chemical Hygiene Plan

which is capable of protecting employees from health hazards associated with hazardous chemicals in that laboratory and ensures that exposures to these chemicals are kept below certain regulated limits. A company's Chemical Hygiene Plan MUST be readily available to employees and employee representatives.

A Chemical Hygiene Plan MUST include each of the following elements and must also indicate specific measures that the employer will take to ensure laboratory employee protection:

- Standard operating procedures relevant to safety and health considerations to be followed when laboratory work involves the use of hazardous chemicals.

- Criteria that the employer will use to determine and implement control measures to reduce employee exposure to hazardous chemicals including engineering controls, the use of personal protective equipment, and hygiene practices.

- Fume hoods and other protective equipment must be functioning properly and specific measures that will be taken to ensure continued adequate performance.

- Provisions for employee information and training.

- Any circumstances under which a particular laboratory operation, procedure, or activity requires prior approval from the employer must be identified.

- The plan must include provisions for medical consultation and medical examinations as required by law.

- Personnel responsible for implementation of the Chemical Hygiene Plan must be identified. Often, this includes assignment of a Chemical Hygiene Officer and, occasionally formation of a Chemical Hygiene Committee.

- The plan must include provisions for additional employee protection for work with particularly hazardous substances. These include "select carcinogens," reproductive toxins, and substances which have a high degree of acute toxicity.

1.1.4 Define oxidizing chemicals.

An oxidizing chemical is one that oxidizes another chemical, the reducing chemical. In doing so, it becomes reduced. This type of reaction is called an oxidation-reduction, or re-dox, reaction. The oxidizing chemical GAINS an electron, while the reducing chemical LOSES an electron.

Common oxidizing agents that may be found in small labs include:
Oxygen gas (O_2), ozone (O_3), halogens (fluorine, chlorine, bromine).
Hypochlorites: such as household bleach, chlorination chemicals.
Nitric acid (HNO_3), Nitrate salts such as sodium or potassium nitrate ($NaNO_3$, KNO_3).
Permanganates and persulfates: such as potassium permanganate ($KMnO_4$).

- For BOD, oxidizers you might use include: bleach, hypochlorite
- For Ammonia, oxidizers you might use include: none
- For Total Phosphorus, oxidizers you might use include: ammonium persulfate
- For Chlorine Residual, oxidizers you might use include: hypochlorite, potassium dichromate

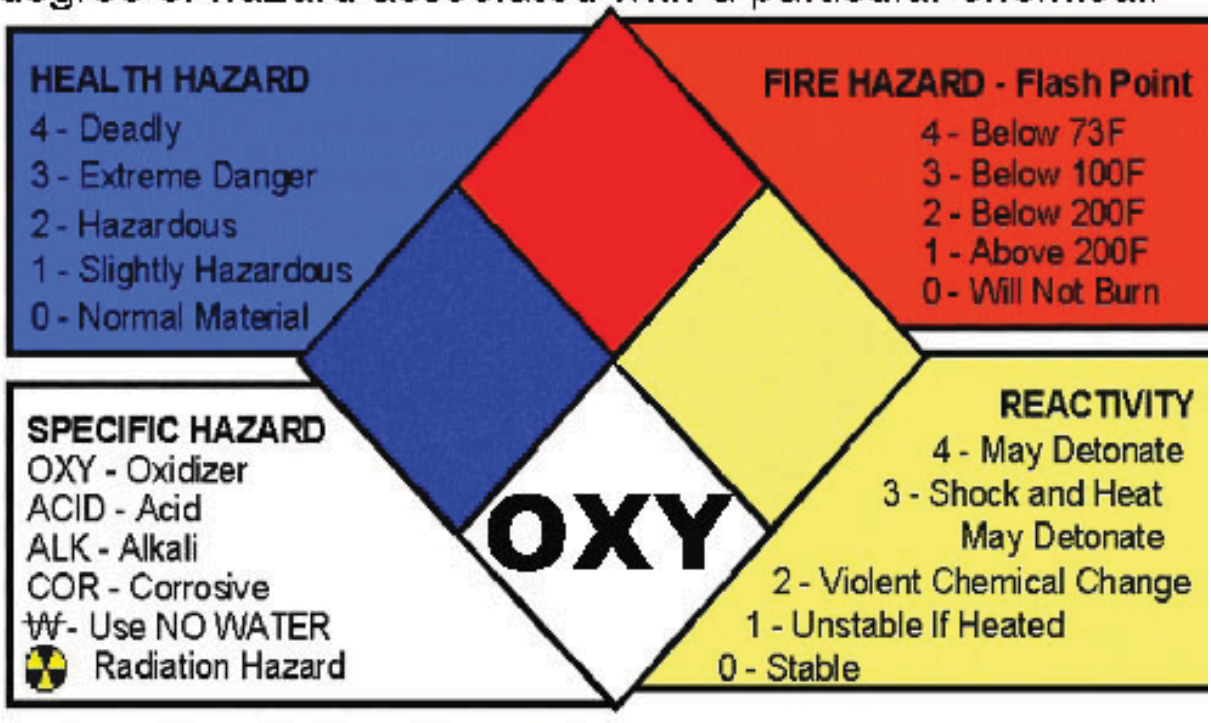
Generally speaking, chemicals whose names end in “-ate” or “-ite” are those that contain a significant amount of bound oxygen, and thus are frequently oxidizers.

There are two main categories of oxidizing agents: (1) reagents that contain an oxygen-oxygen bond and (2) reagents that contain metal-oxygen bonds. Examples of oxidizing agents containing an O—O bond include oxygen gas (O2), ozone (O3), and hydrogen peroxide (H2O2). The most common oxidizing agents with metal-oxygen bonds contain either hexavalent chromium (Cr+6) or heptavalent manganese (Mn+7). Common Cr+6 reagents include chromate (CrO3) and sodium or potassium dichromate (Na2Cr2O7and K2Cr2O7). The most common Mn+7 reagent is potassium permanganate (KMnO4).

NFPA 704 is a standard maintained by the National Fire Protection Association (NFPA) . It defines the commonly named, "fire diamond" used by emergency personnel to quickly and easily identify the risks posed by nearby hazardous materials. The four divisions of the “fire diamond” are typically color-coded, with blue indicating level of health hazard, red indicating flammability, yellow (chemical) reactivity, and white containing special codes for unique hazards. Each of health, flammability and reactivity is rated on a scale from 0 (no hazard; normal substance) to 4 (severe risk). Oxidizers are designated in the white, “special” code area, using a code of “OX” or “OXY”

Figure 1.1.04

The National Fire Protection Association (NFPA 704) system uses a color-coded, diamond-shaped diagram ("fire diamond") of symbols and numbers to indicate the degree of hazard associated with a particular chemical.



1.1.5 Define reducing chemicals.

A reducing chemical is a compound that causes reduction, thereby itself becoming oxidized. Reducing agents remove oxygen from another substance or give hydrogen to it. A reducing chemical is one that reduces another chemical, the oxidizing chemical. In doing so, it becomes oxidized. This type of reaction is called an oxidation-reduction, or re-dox, reaction. The reducing chemical LOSES an electron, while the oxidizing chemical GAINS an electron.

Common reducing chemicals that may be found in small labs include:
Hydrogen (gas), hydrocarbons and their derivatives including alcohols, oils, greases, and organic acids.
Metals (FeCl3, alum, foil).
Ammonia.
Carbon (activated charcoal).

Common reducing agents in the lab include:
In the general lab, reducing chemicals you might use include: isopropyl alcohol, acetone, activated charcoal.

For BOD, reducing chemicals you might use include: sodium bisulfite, sodium metabisulfite, or sodium thiosulfate.

For Ammonia, reducing chemicals you might use include: ammonia.

For Total Phosphorus, reducing chemicals you might use include: none.

For Chlorine Residual, reducing chemicals you might use include: none.

There is no NFPA fire diamond designated for reducers.

Section 1.2 - Chemical Safety

1.2.1 Explain the importance of storing oxidizers separately from reducers.

Mixing oxidizers with reducers can produce violent reactions, even explosions. Generally these reactions are exothermic, which means a great deal of heat is generated. Based on these reactions oxidizers and reducers need to be stored separately.

Chemical incompatibilities to be aware of in the laboratory:
*Acetone - incompatible with acids, oxidizers
*Ammonia - incompatible with acids, bleach (hypochlorite), oxidizers
*Chlorine - incompatible with alcohols, ammonia, combustible materials, flammable compounds, hydrocarbons, hydrogen peroxide, iodine, metals, nitrogen, oxygen, sodium hydroxide
*Hypochlorites - incompatible with acids, activated carbon
*Nitrates - incompatible with acids, nitrites, metals, sulfuric acid
*Nitric acid - incompatible with alcohols, (concentrated) ammonia, organic materials, plastics
*Potassium permanganate - incompatible with sulfuric acid
*Sulfuric acid - incompatible with potassium permanganate
*Special cases (explosive compounds)

Some compounds have reducing groups and oxidizing groups in the same molecule. These tend to be heat and shock sensitive.

Examples:
Chlorites, chlorates, perchlorates, nitrates, e.g. NH_4NO_3. The NO_3^- is the oxidizing agent; the NH_4^+ is the reducing agent.
Organic nitrates and nitro compounds, (e.g., TNT, trinitrotoluene, $CH_3C_6H_2(NO_2)_3$). The nitro (NO_2) groups are oxidizing agents; the carbon atoms are reducing agents.

1.2.2 Discuss safe storage of laboratory chemicals.

- Care should be taken to separate chemicals which are not compatible. This would include those which might react violently or produce dangerous fumes if accidentally mixed.
- Strong oxidizing agents should be stored away from organic solvents or strong reducing agents.
- Acids should be stored in an “acid” cabinet.

- Bases should be stored in a “corrosive” cabinet.
- Flammable solvents should be stored in a “flammable” cabinet.
- Storage cabinets for acids, bases and solvents should be vented to a hood or exhaust system.
- Create and maintain a Chemical Hygiene Plan.

Chapter 2 - Sampling and Sample Handling

Section 2.1 - Sample Collection

2.1.1 Explain how the following sampling errors might affect laboratory results:

-Samples not maintained at proper temperature: Biological activity may change the properties of the sample.

Example: The cold temperature reduces microbial activity. For BOD, it is important to reduce microbial degradation of the sample in order that we obtain a true measure of the samples biochemical oxygen demand..

-Improper or lack of chemical preservation: Biological or chemical activity may change the characteristics of the sample.

Example: without acid preservation for phosphorus, micro-organisms will continue to grow and assimilate phosphorus from the water sample.

-Composite sampler: the sampler is set for infrequent sampling increments. (It would be more representative to reduce sample volume per sample increment, and increase the frequency).

Section 2.2 - Sample Preservation

2.2.1 Explain the significance of the less than or equal to 6°C preservation requirement for samples.

Historically, federal rules regarding temperature preservation stated only that samples must be maintained at a temperature of “< 4°C”. This appeared to be a very firm temperature, with no variance associated with it.

For example, labs are familiar with the acceptable temperature range for drying of TSS samples as 103-105°C (which really translates to 104 ± 1°C), and of the requirement that BOD incubators be maintained at a temperature of 20 ± 1°C. No such flexibility appeared to exist for sample temperature. Consequently a growing national trend established a new requirement that sample temperature be maintained at 4 ± 2°C, or 2 to 6°C. This range clearly identifies that the target temperature for sample preservation is 4°C, but offers a flexible window of compliance.

What the 4 ± 2°C approach does not recognize, however, is that preservation temperatures below 2°C are not unacceptable. In fact, the only limiting criterion for the lower acceptable range for sample temperature is that samples must not be frozen, as freezing samples can change the physical or chemical nature of certain analytes. Consequently, when ch. NR 219, Wisconsin Administrative Code (which governs analysis of wastewater samples) was

revised, this code specifies a temperature of “less than or equal to 6°C” with a footnote that specifies that samples also are not to be frozen.

The most critical thing to remember is that just because the upper limit has been expanded to 6°C, the overall goal of sample preservation has not changed. Therefore, labs should still consider the target sample preservation temperature to be 4°C. If autosamplers or refrigerators appear to be creeping upwards of 4°C, then corrective action should be initiated to provide more cooling to samples. This may include adjusting (and noting in maintenance logs) that the thermostat was adjusted to reduce cooling temperature.

2.2.2 Explain the “15-Minute” rule for sample preservation

Federal regulations state that samples must be either analyzed within 15 minutes or preserved for later analysis if analysis cannot be initiated within that time. Preservation means either temperature or chemical preservation, depending on the analysis. This means that for labs which collect and analyze their own samples, such as wastewater treatment plant labs, samples must be collected and immediately returned to the laboratory where analysis must begin. In some cases, the analysis “begins” by warming the samples up to room temperature. Analyses such as ammonia and total phosphorus require the addition of acid as a chemical preservative. If the analyst plans to immediately begin analysis on the samples, then the acid preservative is not required. However, if the samples will not be analyzed until later in the day, week, or month, then the acid preservative must be added to each sample bottle.

Chapter 3 - Lab Equipment and Instrumentation

Section 3.1 - Definitions

3.1.1 Define colorimeter

A colorimeter is generally any tool that characterizes “color” (as the name suggests) which provides an objective measure of color characteristics. In chemistry, the colorimeter is an apparatus that allows the absorbance of a solution at a specific wavelength (color) of visual light to be determined. This is usually done by preparing a sample according to directions and comparing its color against a reference, or series of references. These comparisons are done visually in some cases, and instrumentally at a fixed wavelength of light in other instances.

White light is made up of many different colors or wavelengths of light. A colored sample typically absorbs only one color or one band of wavelengths from the white light. Different chemical substances absorb varying frequencies of the visible spectrum. Only a small difference would be measured between white light before it passes through a colored sample versus after it passes through a colored sample. The reason for this is that the one color absorbed by the sample is only a small portion of the total amount of light passing through the sample. However, if we could select only that one color or band of wavelengths of light to which the test sample is most sensitive, we would see a large difference between the light before it passes through the sample and after it passes through the sample.

Colorimeters rely on the principle that the absorbance of a substance is proportional to its

concentration i.e., a more concentrated solution gives a higher absorbance reading. Our eyes do not function as spectrometers; they behave like dimmers and switches. We can see shades of darkness and lightness. We see shades of red or green but not both. We see shades of yellow or blue but not both. We can see combinations of red and blue or yellow. We can see combinations of green and yellow or blue. So, to calculate color as the eye sees it, we take the spectra and we convert it to color coordinates called L*.a*.b* or other coordinates that imitate the eye. We can also look at indexes which reduce what we see to one number. For example: yellowness, whiteness, brightness, total color difference. So, if I want to measure color or color difference, I only need a colorimeter. If I want to look at the light physics, I will use a spectrophotometer

3.1.2 Define spectrophotometer

A spectrophotometer is a photometer (a device for measuring light intensity) that can measure intensity as a function of the color, or more specifically, the wavelength of light. A spectrophotometer measures quantitatively the fraction of light that passes through a given solution. In a spectrophotometer, a light from the lamp is guided through a monochromator which allows the user to select which specific wavelength of interest will be passed through the exit slit and into the sample out of the continuous spectrum. This light passes through the sample that is being measured. After the sample absorbs some degree of the light, the intensity of the remaining light is measured with a photodiode (which measures the amount of light, passing through the sample) or other light sensor, and the absorbance for this wavelength is then calculated. See Figure 3.1.02 for key components of a spectrophotometer.

A spectrophotometer gives you spectra: that is information at every wavelength. It can be absorption, reflection, transmission, or other calculations. For the visible, this is usually 320 - 760 nanometer wavelength. Each analyte has a characteristic wavelength that must be used as required by the method employed.

In short, the sequence of events in a spectrophotometer is as follows:
1. The light source shines through the sample.
2. The sample absorbs light.
3. The detector detects how much light the sample has absorbed.
4. The detector then converts how much light the sample absorbed into a number.
5. The numbers are either plotted straight away, or are transmitted to a computer to be further manipulated (e.g. curve smoothing, baseline correction).

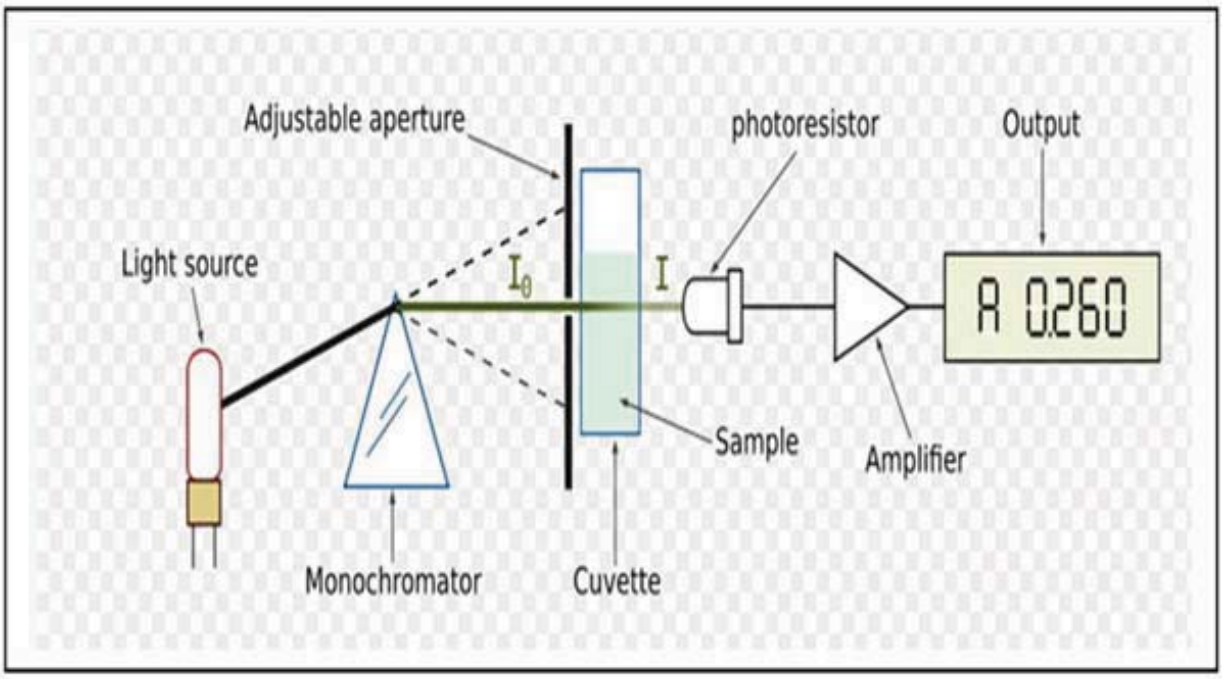
Many spectrophotometers must be calibrated by a procedure known as "zeroing." The absorbance of lab reagent water is set as a baseline value so the absorbance of the sample is relative to reagent water alone. The spectrophotometer then displays absorbance (the amount of light absorbed relative to the analyte concentration).

There are many kinds of spectrophotometers. Among the most important distinctions used to classify them are the wavelengths they work with, the measurement techniques they use, how they acquire a spectrum, and the sources of intensity variation they are designed to measure. Other important features of spectrophotometers include the spectral bandwidth

and linear range. The most common application of spectrophotometers is the measurement of light absorption.

In short, a colorimeter provides an overall measure of the light absorbed, while a spectrophotometer measures the light absorbed at varying wavelengths.

Figure 3.1.02



3.1.3 Discuss differences between colorimeters and spectrophotometers.

Spectrophotometers aren't the answer for every color-measurement application. In cases where precise color measurement is not required, other technologies offer more cost effective quality control. For instance, colorimeters are simpler and less-expensive instruments that use red, green, and blue (RGB) filters to simulate the response of the human eye to light and color. Colorimeters are effective for sorting and for quick in-line checks on less-exacting jobs.

To compare the resolution of a colorimeter with a spectrophotometer, a good visual analogy is this: A colorimeter measures on a scale of inches, while a spectrophotometer measures on a scale of one-sixteenth of an inch.

Colorimeters are appropriate for testing where less accuracy is needed such as chemical addition monitoring (fluoride, chlorine, phosphate) for water supplies. These analyses do not require certification.

Spectrophotometers are appropriate for permit compliance testing such as total phosphorus. Compliance monitoring requires certification.

Section 3.2 - General Labware

3.2.1 Discuss Positive Displacement Pipets

Air displacement pipets reach their limits with high density, viscosity, and vapor pressure liquids. Positive displacement pipets are generally used for difficult liquids such as liquids with high density, viscosity, and vapor pressure. The positive displacement pipets work like a syringe. There is no air-cushion between the disposable piston and the sample. With no elastic air cushion to expand or contract, the aspiration force remains constant, unaffected by the physical properties of the sample. Direct contact allows aspiration of volatile liquids without evaporation. In addition, the absence of air permits rapid pipetting without cavitation. They are expensive because the barrel is replaced as part of the tip. They come in fixed and adjustable, single or multi-channel varieties.

Positive displacement pipets work by having a piston-integrated tip. The piston makes direct contact with the liquid (no air cushion), and there is a positive wiping action of the piston against the capillary walls of the tip which assures dispensing without residual droplets. Direct contact enhances accuracy and precision for liquids which are too heavy or too viscous to be displaced by air.

Positive displacement pipets are the most accurate for pipetting volatile solvents and more suitable for pipetting corrosives and bio-hazardous material.

Positive displacement pipets generally aren't of use in a small wastewater lab.

3.2.2 Discuss Air Displacement Pipets

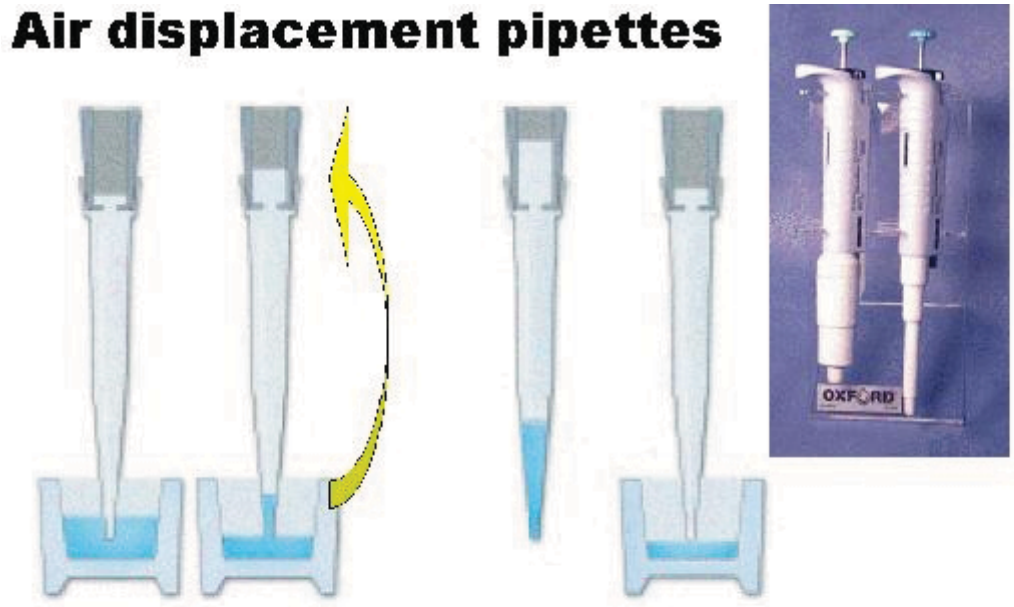
Air displacement pipets operate by piston-driven air displacement. (This is similar to a reciprocating sludge pump [i.e., piston driven]). The plunger is depressed to both draw up and dispense the liquid. Normal operation consists of depressing the plunger button to the first stop while the pipet is held in the air. The tip is then submerged in the liquid to be transported and the plunger is released in a slow and even manner. This draws the liquid up into the tip. The plunger is again depressed in a slow and even manner to the first stop, and then to the second stop, or ‘blowout', position. This action will fully evacuate the tip and dispense the liquid. In an adjustable pipet, the volume of liquid contained in the tip is variable; it can be changed via a dial or other mechanism, depending on the model. Some pipets include a small window which displays the currently selected volume. Note that Hach TenSette® pipettes require the analyst to move the selector to the next highest volume in order to dispense the sample/solution.

Operator consistency is paramount to repeatable operation. In other words use the pipet the same way EVERY TIME. It is critical that the operator develops good pipetting practices. Certain considerations should be observed to ensure maximum accuracy and repeatability:

- When drawing up liquid the tip should be dipped 3 to 5 mm below the surface of the liquid, always at a 90 degree angle.
- When dispensing, the pipet should be held at a 45 degree angle, and the tip placed against the side of the receiving vessel. Glass vessels are preferred; the surface tension of the glass provides additional torsion that results in complete evacuation of the tip.
- The tip must never be wiped off or blotted in any way, even from the exterior, while liquid is

in the tip. These actions tend to bleed off some of the liquid, resulting in decreased accuracy and repeatability.

Figure 3.2.2.1



From:Liquid Handling Application Notebook - Tips on how to pipette
http://www.thermo.com/eThermo/CMA/PDFs/Articles/articlesFile_15449.pdf

3.2.3 Discuss proper maintenance and care of autopipets.

- Perform a general check every 6-12 months, or more frequently, depending how accurate you need to be. The check/service should include re-calibration, greasing of the moving parts and replacement of any worn out seals or other parts. Note that this check is frequency is seperate and distinct from that required for verification of pipet accuracy (3.2.6).
- Check your autopipets daily for damage to the nose of the barrel (where the tip is fitted) or any other obvious damage. If there is a problem, have it serviced because it is unlikely to be fit for the use you need it for.
- Clean your autopipets every day before use - a wipe with about 70% ethanol should do it.
- Store your autopipet vertically, using a specially designed pipet holder. This prevents any liquids that have sneaked into the barrel of the pipet from getting any further inside and corroding it.
- Never put your autopipet on its side with liquid in the tip. The liquid might get in the pipet barrel and cause some serious corrosion damage.
- Use well-fitting tips. Poorly fitting tips allow air to escape when drawing up and dispensing, leading to inaccuracies.

3.2.4 Discuss proper pipetting techniques.

Make sure you know how to pipet properly. The most important rules to follow are:

Pipet with a slow, smooth action.

Hold the pipet vertically when drawing liquid in.

Only immerse the tip slightly when drawing liquid in, otherwise you will coat the outside of

the tip with liquid, which will be transferred along with the volume inside the pipet.

When dispensing the liquid hold the pipet vertically, but keep the sidewall of the receiving vessel at 45 degrees. Pipet against the sidewall or into the liquid that's already there.

3.2.5 Discuss techniques to improve your accuracy in pipetting.

- Pre-wetting. When you dispense liquid from your pipet a coating of the liquid is left on the tip, making the expelled volume slightly less than it should be. Pre-wetting the tip before you pipet will help this. Just draw up the liquid into your pipet then dispense back into the original vessel. The coating is now on the tip so when you now draw up the liquid again and dispense it into the receiving vessel; none of it will be lost to wetting. This is only recommended for volumes greater than 10 microliters.

- Take the ambient temperature into account. Your pipet will have been calibrated at room temperature. If you are working at a different temperature (e.g. in a cold room) your pipet will not be dispensing the displayed volumes.

- Take the sample temperature into account. When repeatedly pipetting cold samples, the first dispensed volume is always larger than expected, but subsequent pipetting with the same tip gave the correct volume. The same was true for hot samples, except that the first dispensed volume was smaller than expected. Their solution was simple - dispense the first volume back into the original vessel, then start pipetting.

- Use a sensible pipet for the volume you want to dispense. The accuracy of your pipet decreases as the dispensed volume approaches the minimum the pipet can handle. So for dispensing 15 microliters, for example, a 1mL pipet would be terrible, a 200 microliter pipet not so good and 20 microliter pipet ideal.

- Use the largest volume possible. Large volumes are easier to pipet accurately than small. Say you are performing an analysis where you have to accurately pipet 5 microliters. Pipetting 5 microliters accurately is not easy and will likely contribute greatly to the statistical error in your results. On the other hand, if you diluted the stock solution 10 times and pipetted 50 microliters, this would be much more accurate, giving you a more accurate measurement.

- How to get maximum accuracy. An analytical balance is more accurate than any pipet. For maximum accuracy, use the pipet to dispense the volume you need but do it into a tared container on a balance. Calculate the actual pipetted volume from mass (volume=mass/density). Of course, this only works for solutions of known density - but for aqueous solutions, which have a density of 1, this is not a problem.

3.2.6 Explain how the accuracy of autopipettors is verified.

Calibration of autopipettors can be done either photometrically or gravimetrically. The photometric approach is rarer due to the need for expensive instrumentation and reagents. The gravimetric approach is what most labs use. This approach assumes that purified water (i.e., lab reagent water) weighs approximately 1.0 gram per milliliter, mL). The exact density of water is based on temperature and can be obtained from a reference table.

Pipets are tested by pipetting consecutive aliquots (specific milliliter volume) of reagent water and comparing the resulting mean and standard deviation of the weight of each aliquot to the nominal weight (based on 1 mL = 1 gm). The accuracy of all pipets must be verified quarterly by analyzing the weights resulting from at least four replicate pipettings. Replicate analyses must meet acceptance criteria or use of the pipet should be discontinued until the problem has been corrected.

Suggested acceptance criteria to use are:
% Inaccuracy:
[(Corr. Mean - true value) ÷ true value × 100] (must be less than 2% and
No single replicate may be greater than 2% from the true value).

%CV:
[(Standard Deviation ÷ Corr. Mean × 100)] (must be less than 1.00)

If you are checking an adjustable volume pipet, at least three different volumes should be tested; 10% of maximum volume, mid volume and maximum volume.

PROCEDURE
1. Place a clean, dry disposable beaker on the balance, close and tare the balance.
2. Apply a clean tip to the pipet.
3. Operate the pipet's action a few times prior to using. This will redistribute the lubricant and ensure a smooth positive action.
4. Wet the tip by drawing up an aliquot of reagent water and discard.
5. Open the balance and pipet an aliquot of reagent water into the disposable beaker taking care to touch off any remaining liquid on the tip.
6. Close the balance. Once the weight has stabilized, record the value on a benchsheet or spreadsheet.
7. Tare the balance and repeat for as many replicates are needed. If a mistake was made during one replicate, repeat using a new replicate.
8. Verify that results fall within the lab's acceptance criteria.

Section 3.3 - Support Equipment

3.3.1 Explain how laboratory thermometers are calibrated.

Each thermometer used in the lab must be assigned a unique identifier such as “BOD incubator thermometer”. Alternatively, the lab can use the serial number etched in the thermometer by the manufacturer.

Thermometers used in the laboratory must be calibrated at least annually against a thermometer traceable to a NIST (National Institute of Standards and Technology) certified thermometer. The NIST thermometer must have been certified within the past five (5) years. Any correction factors associated with the NIST certified thermometer are recorded on a thermometer calibration log sheet and any correction factors associated with the laboratory thermometer(s) are noted on a tag attached to the thermometer. If the liquid column in the thermometer becomes separated, the thermometer is no longer accurate and must not be

used.

The actual calibration is performed by placing the NIST traceable thermometer and the thermometer to be calibrated within the same medium and at the same temperature which the thermometer being calibrated is normally used. Alternatively, the laboratory could use the boiling water/ice bath techniques - all thermometers can then be calibrated at the same time to absolute values. Allow the two thermometers to adjust to the test temperature and then read them at the same time. Document observations appropriately in a logbook indicating what was done and the temperatures recorded. The correction factor is tagged on the calibrated thermometer. For example, if during the calibration procedure within a drying oven the NIST thermometer read 103.5°C and the thermometer under examination read 103.0°C, the thermometer would be tagged in a manner indicating that 0.5°C needs to be added to the observed temperature.

Alternatively, the laboratory may purchase factory certified thermometers traceable to NIST annually. Each thermometer comes with a unique serial number, a certificate of NIST traceability, and the required re-certification and/or expiration date.

Note: Thermometers are calibrated for total immersion or partial immersion. Those calibrated for partial immersion must be immersed only to the depth of the etched circle around the stem of the thermometer just below the thermometer scale readings. Those calibrated for total immersion must be completely immersed in the matrix being measured.

3.3.2 Discuss the process for re-certifying calibration weights.

The weights need to be Class 1 and re-certified at least every five (5) years by an outside metrology service. The re-certification of weights MUST be done by an external vendor (vs. someone that comes into your lab to perform the task) because re-certification of standard weights requires specific environmental conditions (controlled vibration, drafts, temperature, and humidity) that are only available in a certified metrology lab.

This re-certification must be performed sooner than every 5 years if balance checks performed using these weights suggest that a change to one or more certified weights has occurred.

3.3.3 Describe the operating principle of the Beer-Lambert Law.

Because of a special relationship between absorbance and concentration, known as the Beer-Lambert (often referred to as Beer's Law), the concentration of a substance can be determined using absorbance measurement. This relies on the same principles that a student would use to rank a series of concentrations according to the darkness of their color.

For example, if you use a solution of red food coloring in water and measure the amount of blue light absorbed when it passes through the solution, a measurable voltage fluctuation can be induced in a photocell on the opposite side. If now the solution of red dye is diluted in half by the addition of water, the color will be less intense and the voltage generated on the photocell will be approximately half as great. The concentration of a substance in solution

can be measured by calculating the amount of absorption of light at the appropriate wavelength or a particular color.

The Lambert law states that absorption is proportional to the light path length, whereas Beer's law states that absorption is proportional to the concentration of absorbing species in the material. Combining the two laws to form the Beer-Lambert law, which describes how absorbance can be converted to concentration. See Figure 3.3.03A for the Beer-Lambert equation.

Thus, since the molar extinction constant and the cell path are both constant for a given analysis, the equation boils down to: Abs = c. Subsequently, the absorbance for a given analyte will increase as cell path length increases.

The Beer-Lambert law can be used to increase analytical sensitivity (lower detection limit) on occasion. Using phosphorus as an example, the absorbance of a 0.1 mg/L standard, using a typical cuvette with a 1.0 cm path length, is approximately 0.05 absorbance units. If the cell path length is increased to a 5 cm cuvette, the absorbance would be effectively increased five-fold to about 0.250 absorbance units.

Figure 3.3.03A

Beer-Lambert Law

$Abs = \lambda \times b \times c$

Where:
- Abs = Absorbance
- λ = a molar extinction constant, specific to the analyte of interest
- b = the path length (cm) of the cuvette or cell
- c = concentration of the analyte

3.3.4 Explain why pre-programmed calibrations on instruments are not allowed.

A number of commercially available instruments offer "pre-programmed" calibration curves for many of the routine wastewater tests, including chlorine residual and phosphorus. The use of pre-programmed calibrations is unacceptable.

A laboratory must generate its own standard curve. A manufacturer's claim that its method is approved or acceptable does not mean that the approval extends to pre-programmed calibrations. When the EPA extends "approval" to one of these manufacturers that their particular technique is "equivalent" to a referenced EPA method, the approval is granted on the basis of no significant difference in the stoichiometry or chemistry of the procedure.

"Pre-programmed" calibrations establish a fixed relationship between concentration and instrument response. The relationship is formed using new instruments under very controlled conditions by a single analyst. Such an approach does not take into account variables such as instrument maintenance, the lifespan and variability with an aging spectrophotometer bulb, quality and accuracy of reagents and standards, or analyst technique. We all recognize that these variables DO affect the analysis. Therefore a calibration must be performed using the laboratory's instrument, reagents, and personal under the conditions of that laboratory.

Chapter 4 - General Lab Practices

Section 4.1 - Definitions

4.1.1 Define Conductivity

Conductivity or specific conductivity is a measure of a material's ability to conduct an electric current. The ability of water to conduct an electric current is driven by the number of ions dissolved in the water. The more dissolved ions, the greater the conductivity. These "ions" result from the ionization of salts and other chemicals when they become dissolved in water. For example, if you add a pinch of table salt (NaCl) to a liter of deionized water, the salt quickly dissolves. During this process, the NaCl gets broken down into two ionic parts: Na+ and Cl-. It is the presence of these ions in water that causes conductivity.

Drinking water has a conductivity about 100 times greater than that of deionized water. Seawater has a conductivity about 1,000,000 times greater than that of deionized water.

Conductivity is the reciprocal (inverse, or 1 divided by) of electrical resistivity and has the SI units of siemens per meter (S•m-1). The units are typically referred to as "Siemens" however. For most lab applications, conductivity is so low that units of microSiemens (µS) are used. Formerly conductivity was associated with units of "mho", which is ohm, the unit for resistivity (the inverse of conductivity, spelled backwards).

Increasing temperature can make ions in the water move faster. Faster ionic movement leads to increased conductivity. Conductivity levels falsely increase approximately 2% per °C.

Conductivity can be an important tool in the lab as an approximate measure of the amount of dissolved solids in a sample. Because dissolved ions cause conductivity, conductivity has been shown to have a direct correlation to the amount of total dissolved solids (TDS) in a sample. The concentration (mg/L) of TDS in a water sample can be "approximated" by multiplying conductivity by 0.64.

4.1.2 Discuss how conductivity relates to laboratory reagent water quality.

In theory, lab reagent water should be "pure" and thus contain no dissolved solids or ions. Therefore one would expect the conductivity of lab reagent water to be zero.

Pure water is actually a poor conductor.
If water has even a tiny amount of such impurities, then it can conduct electricity much better, because impurities such as salts separate into free ions in aqueous solution by which an electric current can flow.

* Fact:
* The theoretical maximum electrical resistivity for water is approximately 18.2 megaohm-cm at 25 degrees Celsius.
* Electrolytic conductivity (EC) is the inverse of resistivity. Therefore 1/18.2 = 0.055, the theoretical maximum conductivity (µS/cm) of pure water.
* A salt or acid contaminant level exceeding that of even 100 parts per trillion (ppt) [0.1 ppb] in ultrapure water will begin to noticeably lower its resistivity level (RAISING

conductivity)
* An alkalinity of 1 ppm as calcium carbonate ($CaCO_3$) will raise EC to 0.7 µS/cm
* 100 ppb each of sodium and chloride raises EC to 0.45 µS/cm
* Just 25 ppb of NaCl (about 2-3 grains of sand worth) dissolved in purified water will raise the EC to the maximum allowable level for ASTM Type I water

Conductivity gives us a measure of water quality. The ASTM has defined Type I reagent water as water having a maximum conductivity of 0.056 µS/cm at 25°C. ASTM "Type II" water has a maximum conductivity of 1.0 µS/cm at 25°C. Conductivity means ions are present and the presence of ions clearly means that the water is not "pure". Conductivity is useful as an indication that ion exchange resin is overloaded, that a reverse osmosis membrane has been breached, or simply that your reagent water may not be of sufficient quality for use in testing.

The drawbacks to using conductivity alone as a means of verifying water quality are:
1. Conductivity ONLY measures substances that ionize…i.e. form ions. You can dissolve 1000 ppb of sugar in pure water and still not exceed ASTM Type I water criteria for conductivity.

2. It is virtually impossible to measure conductivity accurately to Type I or Type II levels without a closed system and VERY sensitive conductivity equipment. The nominal levels of CO_2 in the atmosphere will cause gaseous CO_2 to enter pure water causing a chemical reaction which increases conductivity.

The theoretical conductivity in pure water with addition of CO_2 is approximately 0.8 µSiemens. Therefore, just by exposure to air, lab reagent water will not be able to meet the requirements of ASTM Type I water. Lab reagent water criteria specified in Standard Methods and for the EPA's Safe Drinking Water program (2 µS at 25°C) are more realistic targets.

Section 4.2 - Measurement Techniques

4.2.1 Discuss cell/cuvette quality control.

Cuvettes are designed to transmit light without any reflection or refraction. Any scratches, smudges, or even chemical film on the cuvette will affect light transmission, affecting the analysis. Though you may not be able to see it with the naked eye, two cuvettes that appear clean can have two very different light transmission abilities. You can confirm this by zeroing a spectrometer with one clean cuvette containing only lab reagent water and then placing a second cuvette containing only lab reagent water and measuring absorbance. The second cuvette SHOULD read zero absorbance. An absorbance greater than zero means that the cuvette is dirtier or has fine etchings on it than the first cuvette. Similarly, if a negative absorbance is obtained, this means that the second cuvette is of better quality/cleanliness than the first.

When using cuvettes, always ensure that dirt or grease from fingers is not affecting the pathway of light. Use clean cuvettes. Clean the outside of the cuvette with clean lab quality tissue paper. Do not clean cuvettes with a wire brush; use a soft plastic brush or a cotton Q-

tip. Also make sure that the manufacturer's alignment mark on the cuvette is lined up squarely in front.

Some cuvettes have ridges or are opaque on opposing sides for finger grip and optically clear sides that are used for measurement. The optical sides must be kept clean. If cuvettes become stained or scratched, replace the cuvette.

4.2.2 Identify the causes and corrective action to eliminate air bubbles or liquid clinging to the side of a buret or pipet.

Causes: Air bubbles or liquid clinging to the side of a buret or pipet are caused by dirty or greasy glassware.

Correction: The remedy is cleaning the glassware effectively. Air bubbles that may form around the stopcock of a buret can be removed by shaking or tapping the buret, or, by passing a small diameter wire up through the area.

Section 4.3 - Reagent & Standard Preparation

4.3.1 Discuss the C1 V1 = C2 V2 formula.

See Figure 4.3.01 for a more visual means of understanding and properly applying the C1V1=C2V2 formula.

C1V1 = C2V2

C1 = Concentration of original solution
C2 = Concentration of final (diluted) solution
V1 = Volume of original solution
V2 = Volume of final (diluted) solution

Basic Rules for Solving C1V1 = C2V2 Problems:
(1) 3 of the 4 values must be known.
(2) The units of volume and concentration must be the same respectively.
(3) Either C1V1 or C2V2 must be known and it must be clear which is which.
(4) Any unit of volume or concentration may be used.

Tricks to understanding C1V1=C2V2:

C1v1 = c2V2 (note use of UPPERcase vs. lowercase)
C1v1 >< c2V2 (replace the "=" sign with "><")

C1 > c2
The first greater than sign indicates that the initial concentration, as also indicated by uppercase "C", is greater than the concentration (lowercase "c") of the diluted sample .

v1 < V2
Similarly, the less than sign indicates that the initial volume, as also indicated by lowercase "v", is less than the volume (uppercase "V") of the diluted sample.

The concentration of the original solution is always greater than that of the final (diluted) solution. And the final solution is larger in volume.

Logic: when we dilute we ADD water, thereby, increasing the volume and decreasing the concentration.

EXAMPLE: You have a 50 mg/L Phosphorus stock standard [C1, concentration of original solution]. You want to make a 'working' solution of 2 mg/L [c2, concentration of the diluted solution] from which to prepare calibration standards. You want to make 100 mLs [V2, volume of diluted solution] of this 'working' standard.

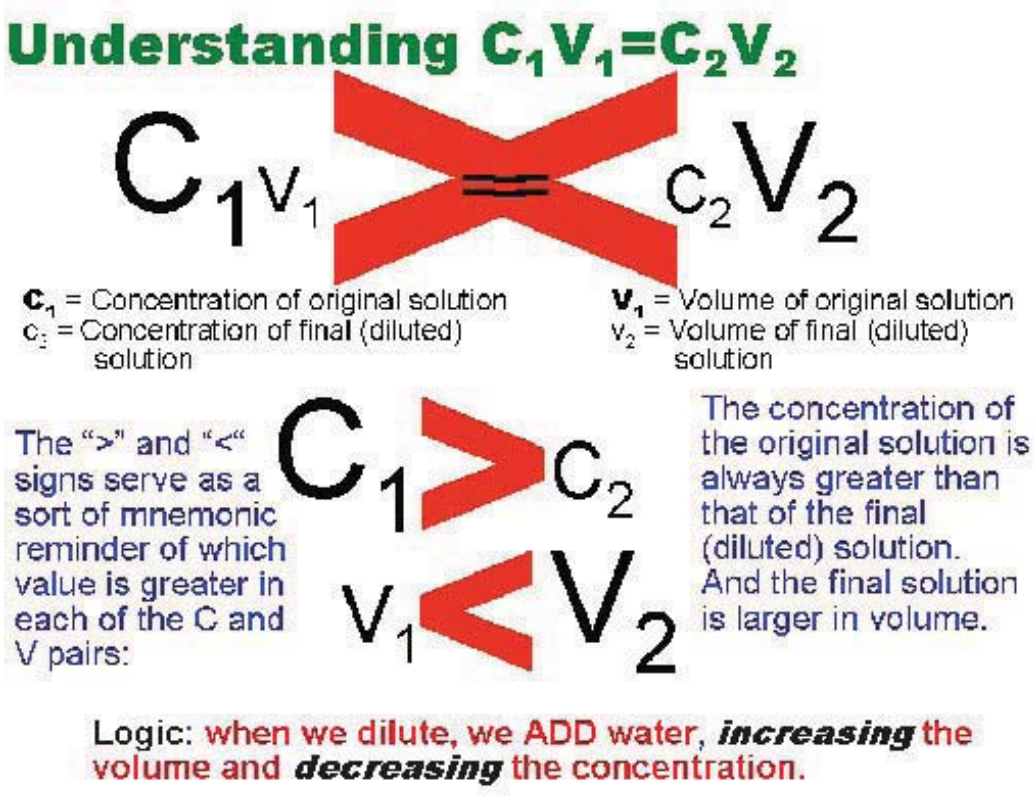
C1 > c2 …so 50 mg/L must be C1

C1v1 = c2V2

C1 = 50 mg/L c2= 2 mg/L
v1 = ? mL V2= 100 mL

(50 x v1) = (2 x 100)
(50 x v1) = 200 [Divide both sides by 50!!!]
v1 = 200 ÷ 50
v1 = 4 mL

Figure 4.3.01



Section 4.4 - Reagent Water

4.4.1 Discuss deionization as a means to produce reagent water.

Deionization is a method used most often by laboratories to produce purified water on-demand and is able to purify water to a maximum resistivity of 18.2 megohm/cm at 25ºC. A deionization system usually consists of one to four cylindrical cartridges hooked up to plumbing and hanging on a wall near a sink. While it doesn't produce absolutely pure water, it is convenient, quick, and may be sufficient for many applications. It is an excellent system for removing dissolved solids and gases, although it has a generally poor rating for other impurities.

In deionization two types of synthetic resins are used, one to remove positively charged ions (cations) and another to remove negatively charged ions (anions). Cation deionization (DI) resins remove cations, such as calcium, magnesium, sodium, and ammonium, replacing them with the hydrogen (H+) ion. Anion deionization resins remove anions, such as chloride, nitrate, nitrite, sulfate, and bicarbonate, replacing them with the hydroxide (OH-) ion.

Deionization resins have design capacities and must be regenerated and/or replaced upon exhaustion. This occurs when equilibrium between the adsorbed ions is reached. Cation deionization resins are regenerated by treatment with acid, which replenishes the sites with H+ ions. Anion deionization resins are regenerated with a strong base which replenishes (OH-) ions.

The two basic configurations of deionization systems are two-bed and mixed-bed.

Two-bed deionization systems have separate tanks of cation and anion resins. In mixed-bed deionization systems, the anion and cation resins are blended into a single tank or vessel. Generally, mixed-bed systems will produce higher quality water with a lower total capacity than two-bed systems.

Deionization can produce extremely high-quality water in terms of dissolved ions or minerals, up to the maximum resistance of 18.2 megohms/cm. However, they do not generally remove organics and can become a breeding ground for bacteria, actually diminishing water quality where organic and microbial contamination is critical. Failure to regenerate the deionization resins at the proper time may result in harmful salts remaining in the water or even worse, being increased in concentration. Partially exhausted deionization resin beds can increase levels of some dangerous contaminants due to the resin’s selectivity for specific ions, and may add particulates and resin fines to the deionized water.

With distillation, volatile impurities such as chlorine, carbon dioxide, silica, ammonia, and a variety of organic compounds will 'carry over' into the distillate. DI systems effectively remove ions, but they do not effectively remove most organics or microorganisms. Microorganisms can attach to the resins, providing a culture media for rapid bacterial growth. These microbes slough off and can be present in the purified water. Additionally, without proper maintenance, resin particles can also be found in deionized water. Consequently, if ultra-pure water is desired, both deionization and distillation require the use

of an additional “polishing” step. For distilled water, carbon filtration is typically employed. The carbon (which itself can eventually become spent) traps volatile contaminants like chlorine or organics. Ion exchange should be used with a final filter if particle-free water is required.

Deionization advantages:
- Removes dissolved inorganics effectively.
- Regenerable (service deionization).
- Relatively inexpensive initial capital investment.

Deionization disadvantages:
- Does not effectively remove particles, bacteria or bacterial byproducts.
- DI beds can generate resin particles and culture bacteria
- High operating costs over long-term.

4.4.2 Identify the laboratory tests affected if the following problems occur with laboratory reagent water:

HIGH COPPER (CU) OR CHROMIUM (CR) LEVELS
Copper and chromium are quite toxic to organisms. High levels of either could significantly affect any biological tests (BOD, fecal coliform) by inhibiting biological growth.

DISSOLVED BIODEGRADABLE SOLIDS
Any dissolved solids which are biodegradable will cause high blank depletions in the BOD tests, and may affect the fecal coliform test as well. It could affect the ammonia nitrogen test by shortening the life of ion exchange columns used to make ammonia-free water.

HIGH CONDUCTIVITY
Elevated conductivity is an indication of an increase in dissolved ions in the water. Some of these can be interfering substances, such as, copper, chromium, or ammonia. The more substances which are dissolved in the theoretically "pure" lab reagent water, the more likely it will be to have interferences in the tests. Elevated levels of trace metals could affect BOD results, while elevated ammonia levels will impact ammonia test results.

Chapter 5 - Lab Analysis

Section 5.1 - Definitions

5.1.1 Define supersaturation.

Supersaturation means that the water contains more dissolved oxygen (DO) than it SHOULD contain according to physics. According to tables, the saturation point of oxygen in water at 20°C and 760 mm pressure --standard temperature and pressure at sea level-- is 9.06 mg/L. So, yes, at sea level and 20°C, anything over 9.06 mg/L represents supersaturation.

In Wisconsin, altitudes typically are about 1000 ft above sea level, and standard pressure drops to about 734 mm (pressure drops about 26 mm of Hg for every 1000 feet of altitude). Therefore, at 20°C and 734 mm pressure, DO saturation falls to 8.76 mg/L. Under these

conditions, DO values greater than 8.76 mg/L represent supersaturation. If a lab is warmer than 20°C, the altitude is higher than 1000 feet above sea level, and there is a low pressure system in effect, saturations can be much lower.

The bottom line is that the method infers that supersaturation is anything above 9.0 mg/L. However, in reality saturation will vary with temperature and pressure. Consult a DO saturation table.

5.1.2 Define pH.

The pH is a range of numbers expressing the relative acidity or basicity of a solution. Mathematically, the pH value is the negative logarithm of the molar hydrogen-ion concentration in a solution.

pH = -log [H+]

Since the scale is logarithmic, the pH changes by one for every power of ten change in hydrogen-ion concentration.

pH meters typically employ a probe with a glass electrode. The probe contains an acidic aqueous solution enclosed by a special glass membrane that allows migration of hydrogen-ions. The electromotive force generated in the probe is linearly proportional to pH. A selection of buffers of a known pH are used to establish this linear relationship and sample pH is interpolated.

Pure water dissociates to yield equivalent concentrations of hydrogen [H+] and hydroxide [OH-] ions:
H2O--> [H+] + [OH-]
The equilibrium for pure water is [H+] x [OH-] = Kw = 10^ -14
=10^ -7 x 10^ -7
Therefore, at equilibrium [H+] = [OH-] = 10^ -7 = pH of 7
The scale ranges from 0 to 14 [H+] = 10^0 to 10^ -14

Section 5.2 - Biochemical Oxygen Demand (BOD)

5.2.1 Discuss the testing differences between BOD and cBOD.

The only difference between samples analyzed for BOD and those analyzed for cBOD is the addition of a chemical inhibitor to all samples for which cBOD is determined.

In the absence of nitrogenous demand and nitrifying organisms, BOD and cBOD values should be equivalent. This is because the inhibitor theoretically suppresses only Nitrosomonas sp., the microorganism which is responsible for the first reaction in the nitrification bio-chemical reactions. In a sample in which no nitrification is expected to occur, adding the inhibiting agent should not change the results, thus explaining why, in these cases, BOD and cBOD would be expected to be equivalent.

In practice, however, a low bias has been reported for cBOD results relative to BOD results, when nitrification would not be expected. This may be due to a toxic affect that the inhibitor

agent has on microbial species other than Nitrosomonas.

5.2.2 Discuss how to determine the proper sample volumes for the BOD test.

There are tables and charts that have been developed to assist analysts in making the best dilutions for any given sample. See Figure 5.2.02 for a chart developed by chemists at the State Laboratory of Hygiene, to help analyst choose proper volumes for BOD analysis.To use these charts, however, the analyst needs to have some idea of the sample BOD. All of these tables work off of a simple concept:

1. Under typical conditions, at saturation in Wisconsin, initial DO should be about 8.5 mg/L.
2. The FINAL DO cannot be less than 1.0 mg/L.
3. Therefore the working range of DOs for any dilution is about 7.5 mg/L.

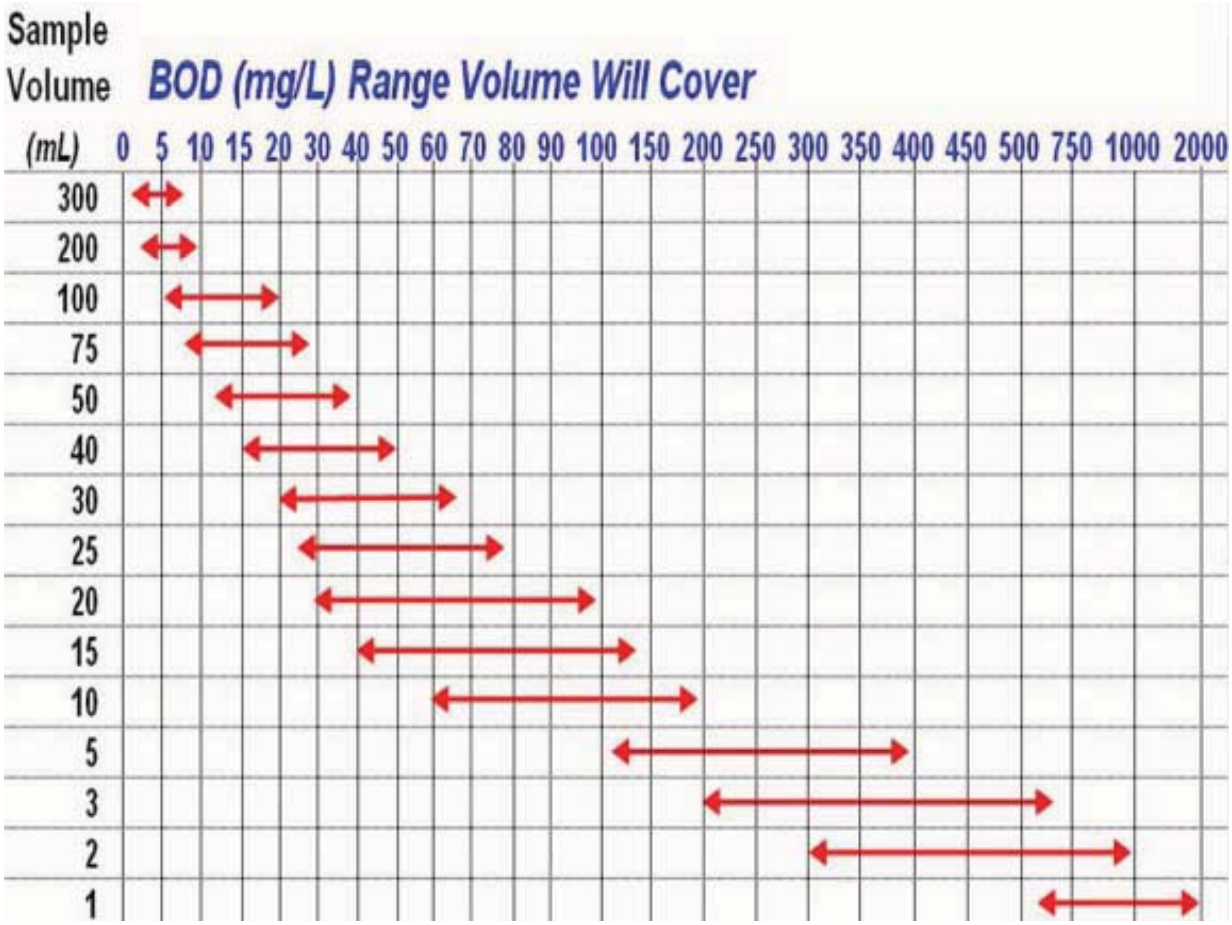
To determine optimal dilution, divide the expected BOD of the sample by 7.5. For example, if you have a very clean effluent and BOD is typically 5 - 10 mg/L, then 5 divided by 7.5 = 0.7 and 10 divided by 7.5 = 1.3. Therefore your optimal dilution factor is between 0.7 and 1.3. The middle of this range is a dilution factor of 1.0. Now divide the maximum volume of sample in a BOD bottle (300 mLs) by that dilution factor. 300 mLs divided by 1.0 = 300 mLs. Therefore, the BEST dilution for a sample with an expected BOD of 5-10 mg/L is 300 mLs.

Typically you would then use one dilution using slightly more volume and one dilution of slightly less volume, to bracket the expected BOD range. Since this sample requires a full bottle, you can't use MORE sample volume. Therefore, a second dilution of about 250 mLs is appropriate. Note that 300 divided by 250 represents a dilution factor of 1.2.

Now consider an influent wastewater sample which typically ranges between 150 and 250 mg/L for BOD. 150 divided by 7.5 = 20 and 250 divided by 7.5 = 33 (round it to 30) . The best dilutions for this sample would be using dilution factors of 20 to 30. A dilution factor of 20 means a sample volume of 15 mLs, and a dilution factor of 30 means a sample volume of 10 mLs. Therefore use sample volumes between 10 and 15 mLs.

In the absence of prior knowledge, use the following guidelines for dilutions:
- strong industrial wastes: < 3 mLs of sample (<1% dilution)
- raw and settled wastewater: 3 - 15 mLs of sample (1 - 5% dilution)
- biologically treated effluent: 15 - 75 mLs of sample (5 - 25% dilution)
- polluted river waters: 75 - 300 mLs of sample (25 - 100% dilution)

Figure 5.2.02



5.2.3 Discuss how the quality of dilution water affects the BOD test.

The quality of the dilution water can affect the BOD test. The source of dilution water is not restricted and may be distilled, tap, or receiving-stream water free of biodegradable organics and bio-inhibitory substances such as chlorine or heavy metals. Distilled water may contain ammonia or volatile organics; deionized waters often are contaminated with soluble organics leached from the resin bed. Use of copper-lined stills or copper fittings attached to distilled water lines may produce water containing excessive amounts of copper.

If the dilution water is of poor quality, the BOD of the dilution water will appear as sample BOD. This effect will be amplified by the dilution factor. A positive bias will result if dilution water quality is compromised.

5.2.4 Discuss potential causes for a positive bias.

Some potential causes for a positive bias are:

- If you are aerating the dilution water with a lab air line, sometimes dirt and grease can come through the line into the water (from the compressor). It only takes a small amount to cause the water to be affected giving you a high control check.

Inline air filters can help this problem. Or aerating without a compressor using a vacuum setup.

- The quality of your dilution water may not be caused from contamination. How much dissolved oxygen is in your dilution water at test start-up? More than saturation? Do you set up on a cold bench top during the winter? This can cause your dilution water to drop in temperature and thus retain more oxygen. If you see lots of bubbles clinging to the inside of the bottles there is a need to discharge them. Any excess oxygen must be shaken out of the dilution water to obtain valid results that give you usable data.

- Does your lab experience large temperature changes during set-up and prep times? If your lab has as much as a 5°C temperature change during the day, the meter would get calibrated first thing along with prepping the dilution water and seed. By the time the actual test set-up began the meter's calibration most of the time would be lost. You need to recalibrate just before starting the test.

- If you are on municipal water, check the total residual chlorine on your tap water. Cold water retains chlorine much longer than warm water. Some waters can have a residual of as much as 1.0 mg/L. Some hot tap water can even have a residual. To solve this problem you need to increase the number of DI rinses of the bottles during the winter when water temperatures are colder.

- If you use a vacuum pump to aerate your dilution water, look around your lab for evidence of dust. Dust will get sucked into the intake of the pump and it will be "pumped" into your water. If you do anything else in the lab that creates fumes, odors, etc., then you probably need an activated carbon filter attached on the intake side of the pump to sweep the contaminants out of the air.

- Any contamination in the water will cause problems with the test. Expensive deionized water systems often leach organic compounds from the ion-exchange resins or allow bacteria to grow on the resins which will give high bias, even though standard conductivity measurements show the quality is very good.

5.2.5 Discuss how oxygen saturation can be a potential cause for a postive bias.

Oxygen saturation varies ± 0.3 mg/L with normal pressure range. Remember: you calibrate on day 0 and again on day 5. What if samples go in under a low pressure, but come out under a high? Conversely, what if samples go in under a high pressure, but come out under a low?

We know that water can hold more oxygen as pressure increases. So, what happens if on Day 0 when you calibrate your meter, it’s a sunny, but bitter cold day under a very high pressure system? Suppose the barometric pressure in your lab is 29.53 inches (750 mm) and the lab temperature is a steady 20 °C. Water at this temperature and pressure can hold (is saturated at) 8.94 mg/L oxygen. Five days later, when you take samples out of the incubator, a winter blizzard is underway outside, and the barometric pressure has dropped to 28.35” (720 mm). Under these conditions, the oxygen saturation

point of water is 8.58 mg/L.

Assume for a moment that your dilution water is contamination free, and thus no change in the DO from the initial reading is expected. Unfortunately, the blanks were saturated initially with 8.94 mg/L DO and now these samples are supersaturated. What happens to the 0.36 mg/L of oxygen by which the blanks
are over-saturated? Oxygen does not like to be supersaturated and it doesn't take much for any excess to dissipate from the system. Occasionally you will see that bubbles have formed in the BOD bottle; remove the cap and the oxygen quickly dissipates before the DO in the blank is measured. This can result in a perceived DO depletion of 0.36 mg/L, more than enough to "fail" the method specified criteria for blanks.

5.2.6 Discuss potential causes for a negative bias.

Conversely, you may notice a negative bias. A negative bias may underestimate the sample BOD results. Some potential causes for a negative bias are:

- Micro-bubbles in the BOD bottle from the dilution water. This can happen when the blank has small bubbles that are difficult to see when the bottle is initially set. This is a difficult problem to diagnose 5 days later because small micro-bubbles that might have been in the bottle when it was set might be fully dissolved by the time that bottle has been read. As a result one might not see anything out of the ordinary, but when the measurement is performed there is a higher DO at the end of the 5 days than there is at the beginning. This results in a low BOD blank.

- Incorrect DO measurement. This can be caused by an incorrect calibration,
either before (when setup), or after the incubation period. This can lead to a slight off calibration (on the low side), initially resulting in an apparent gain (increase) in oxygen in the blank. If this happens routinely, and if it is severe (less than -0.20 mg/L), then it probably indicates a severe problem with the measurement system.

- Incorrectly seated stopper. The BOD bottle stopper must be "seated" or low
blanks will be a possibility. Specifically if the water temperature is too high to begin with (even 21 degrees is high) and the stopper is simply "dropped in" oxygen exchange at the interface can lead to negative blanks. Some analysts think that the initial water temperature is the issue and so become obsessed with having exactly 20 degree water for the incubation water. The simpler and easier solution is to gently twist and seat the stopper in each water bottle and insuring the complete lack of bubbles in the bottle after sealing. Also, if BOD bottles do not have a proper water seal while incubating, DO values could drift up slightly if the water was somewhat under saturated to begin with, causing blanks to suddenly drop.

- A sudden drop in a blank may mean that a negative bias has crept into your system. While a bad DO probe generally drifts down, not up, it's possible that the error could be there. Otherwise an unlocked cal adjust knob might have gotten knocked out of whack.

5.2.7 Discuss a procedure to identify the cause of toxic dilution water, or organic contaminants in dilution water.

TOXIC DILUTION WATER

First of all, what is meant by "toxic dilution water"? The DO depletion of dilution water after 5 days incubation must be below 0.20 mg/L, and it would seem that any low value would be good. But what happens if the blank is -0.05 mg/L (i.e., the blank appeared to GAIN oxygen)? What does this mean? Obtain water from suitable source—distilled, tap, or receiving water. Make sure the water is free of heavy metals (specifically copper) and toxic substances, such as chlorine, that can interfere with BOD measurements. To determine the cause, use the following procedures:

1. Check for chlorine residual - Depending on your source for dilution water, be sure to check for chlorine residual or any reaction with DPD that might indicate an oxidant.

2. Check for heavy metals contamination - Never use copper-lined stills. Distilled water may be contaminated by using copper-lined stills or copper fittings—obtain from another source. Protect source water quality by using clean glassware, tubing, and bottles.

3. Obtain a different source water for making up your dilution water and check response.

4. Toxic Seed - Sometimes the seed has toxic compounds in it that inhibits the growth of the microbes. When the growth is inhibited, there will naturally be less oxygen demand than there would be if the microbes were not inhibited by toxics. The best way to detect the toxicity of the seed is to plot the oxygen uptake versus the mL of seed that is used. If the uptake is not linear, then there is toxicity in the seed. The only choice at this point is to get rid of the toxic seed and try another seed.

5. Narrowing down the cause could be done by having a water suitability test run by an outside laboratory.

ORGANIC CONTAMINANTS IN DILUTION WATER

Deionized water may contain detectable amounts of ammonia, volatile, or semi-volatile organic compounds. Deionized water is sometimes a problem because the resin cartridges themselves will release organic compounds into the water. These compounds are undetectable by conductivity measurements, and conductivity measurements are one of the main ways that purity of deionized water is determined.

Possible remedies included:
- Increase purity of dilution water or obtain from another source.
- Age water for 5-10 days before use.
- Use an activated charcoal as a polishing unit.

If there is a reservoir or storage bottles for the water system, inspect the reservoir or storage bottles for signs of microbiological growth. If there is any evidence of microbial growth clean the reservoir and/or storage bottle. Even if there is no sign of growth, consider cleaning the

reservoir/storage bottle.

- Check your water by analyzing it for Total Organic Carbon (TOC). If the TOC exceeds 200 µg/L then replace the resin cartridges.
- Use deionized water that has been passed through mixed-bed resin columns.
- Obtain water from another laboratory or a retail store (steam distilled is best) and use that water for blanks. If the blank problem goes away then the problem was definitely the water.
- Organic contaminants can be detected by the KMnO4 color retention test, or by analysis of total organic carbon. The laboratory water should be tested at each point of treatment or container change. If the problem is in the feed water, treatment with carbon filters may handle it.

5.2.8 Discuss factors that would result in excessive DO depletion in blanks.

(1) CALIBRATION
The single greatest cause for blank “failures” (DO depletion greater than 0.2 mg/L) stems from calibration problems.

Blank depletions due to calibration errors generally tend to be SMALL effects (depletion of 0.2 - 0.5 mg/L)

The effect can be either LOW or HIGH bias (blanks deplete > 0.2 mg/L or GAIN > 0.2 mg/L). Since it violates laws of physics to gain oxygen, and if the final DO is greater than the initial DO, this is nearly always a sign of calibration errors.

The basic problem is that errors in calibration cause the initial DO reading to be biased high (or the final DO reading is biased low). The net result is that it appears to be a DO depletion.

(2) SUPERSATURATION
If the initial DO of the blank is above the saturation point, all of this DO will come out of the solution during incubation (sometimes seen as micro-bubbles just underneath the bottle stopper.) This appears to be depletion, but it is actually degassing.

(3) CONTAMINATION (organic matter + micro-organisms)
Contamination, when it occurs, tends to be LARGE effect (i.e. DO depletions of > 0.5 mg/L).

Contamination problems will ALWAYS result in excessive depletions.

Note that contamination from organic material or micro-organisms alone will NOT cause an exceedance in blanks. There must be contamination from BOTH organic matter and microorganisms. Without the presence of microorganisms, there is nothing to break down the waste material and thus no oxygen will be utilized. Even if there is microbial contamination, without the presence of waste material, there is nothing for the microorganisms to break down and thus no - or minimal - oxygen will be utilized. Be aware that over-engineered water purification systems can result in insufficient water utilization creating a stagnancy within the water system. This can become a breeding ground for

microbes, and thus the use of water from a purification system may be the cause of failures.

5.2.9 Discuss what is meant by nitrification.

Nitrogenous oxygen demand (NOD) is the amount of oxygen used during the breakdown and conversion of organic nitrogen and inorganic nitrogen forms. This process is called nitrification. We are typically concerned with the inorganic forms, and specifically, ammonia. This means that if the wastewater contains ammonia (and some do, particularly lagoons) AND nitrifying bacteria are present, then oxygen can be used during the conversion of ammonia in to nitrate and nitrite. This oxygen used up is measured as BOD, leading to BOD results biased high. The key equations are as follows:

Reduced Nitrogen (NH_3) + Oxygen (O_2) --> Nitrite (NO_2) + O_2 --> Nitrate (NO_3)

NH_3 + 1.5 O_2 + Nitrosomonas bacteria --> NO_2
NO_2 + 0.5 O_2 + Nitrobacter bacteria --> NO_3

NH_3 (ammonia) + 2 O_2 (oxygen) --> NO_3 (Nitrate)

So… 1 mole of nitrogen (as NH_3) reacts with 2 moles of oxygen (as O_2). Since 1 mole of nitrogen weighs 14 amu and 1 mole of oxygen weighs 2.282 amu, then we have a reaction ratio (N:O_2) in terms of mass (or concentration) of 1:(2 x 2.282) or 1:4.57.

Theoretically, then, 1 mg/L of NH_3-N requires 4.57 mg/L O_2 to oxidize NH_3 to NO_3--N. Why is this important? We have to remember that our dilution water contains ammonia!!! That means that even if the SAMPLE contains no ammonia or nitrogen forms, but nitrifying organisms ARE present, we can have nitrogenous demand adding to the BOD.

NH_3-N in dilution water can contribute up to 1.9 mg NOD x dilution factor to a BOD sample. Thus a 200 mL sample yields 1.9 mg/L x (300 / 200) or 2.85 mg/L BOD.

If your facility experiences Nitrogenous Oxygen Demand you should consider analyzing cBOD rather than BOD. Consult your DNR Basin engineer.

5.2.10 Discuss the effect on the results of a BOD analysis if the incubator temperature was outside method requirements.

If an incubator temperature were changed to 22°C during the BOD test, the following effects could happen:

* All biological reactions would be more active, and possibly more oxygen would be used.

* The blanks which contained oxygen close to the saturation point 20°C could be supersaturated at 22°C. Bubbles would form in the bottles. After 5 days, oxygen could evolve as soon as the cap was removed leading to an apparent high blank depletion.

If an incubator temperature were changed to less than 20°C (e.g., 18°C) during the BOD test, the following effects could happen:

* Biological reactions would tend to be less active, which would likely result in less oxygen utilization (than would be expected at 19-21°C). This would theoretically result in low bias in results.

* Blanks which contained oxygen close to the saturation point at 20°C would be under-saturated at 18°C.

* Without a liquid seal around the BOD bottle caps, the cooling of the samples in the incubator could result in a vacuum effect drawing oxygen into the bottles, leading to an apparent increase in oxygen in blanks.

5.2.11 Outline a troubleshooting procedure to determine the cause of an apparent blank depletion.

Generally, begin by eliminating the items easiest to check, including the following:
- Check the DO meter calibration
- Check for possible problems with the set-up or initial DO reading. Check if the dilution water is too cold, or supersaturated. Check to see if the initial DO is higher than the saturation value for that temperature.
- Check the incubator temperature log. Check to see if the laboratory itself was hotter or colder than 20°C, if it was, the incubator may not have been able to handle the extremes. A maximum/minimum thermometer could verify this if there is a question.
- Check the maintenance record on the water system and DO meter.
- Check the bottle cleaning procedure.
- Check for growth in the delivery tube as evidenced by discoloration.
- Check for contaminated nutrient solutions -- any visible floc, or if the age is excessive.
- Check for recording errors.

5.2.12 List the possible causes of INCREASED dissolved oxygen in a dilution water blank.

- There are small air bubbles initially in the BOD bottle which subsequently dissolve into solution, adding to the bottle's dissolved oxygen.

- Operator error during calibration of the dissolved oxygen meter.

- The accuracy of the calibration procedure itself may be the cause. If air calibration without barometric pressure correction is the method being used to standardize the meter, the standard calibration error of the meter is increased. Under the best of circumstances, precision of dissolved oxygen meter calibration is ± 0.05 mg/L. Therefore, a slight dissolved oxygen increase could simply be the result of normal error of meter calibration.

- Lack of a water seal on the neck of the BOD bottle.

5.2.13 Explain the potential reasons why GGA results could be unacceptably high.

HIGH BIAS IN GGA
(1) Nitrification
Seed source selection is critical; if the plant process includes recycling final effluent into primary clarifiers, you could be adding nitrifying organisms to the seed (if you use primary

effluent as seed material). To determine if nitrification is occurring, try adding a nitrification inhibitor. Compare GGAs seeded with domestic wastewater vs. commercial (Polyseed, BOD seed). If nitrification is occurring, select another source (that does not receive final wastewater) or use a commercially obtained synthetic seed.

(2) Cold GGA solution
If you don't warm the GGA to room temperature (20 ± 3°C) before use, results will be consistently high.

(3) Contamination - organic matter
The contamination is likely "dirty glassware", providing a food source. Your blanks may even meet depletion criteria because -despite availability of a food source (the "crud") - there is no source of bugs and therefore no oxygen can be used. GGAs will typically fail high due to the extra oxygen consumed by the bugs as they attack both the GGA and the "crud".

Contamination can also result from insufficient rinsing of the DO probe after measuring highly concentrated samples.

(4) Contamination - Microorganisms ("bugs")
The contamination source may be from "bugs" in the lab reagent water, possibly from a bad filter in a DI system. As long as your glassware is clean, blanks will meet depletion criteria. If there is no "food source" (e.g., "crud" on the glassware) to keep bugs going and expending oxygen, GGAs will generally fail high due to the extra oxygen consumed by the bugs as they attack the GGA

Contamination, when it occurs, tends to be a LARGE effect (i.e. DO depletions of > 0.5 mg/L).

NOTE: Contamination from either "bugs" or BOD material alone will cause high bias in GGA but is not likely to cause an exceedance in blanks. There must be contamination from BOTH "bugs" AND waste material for contamination to result in blank exceedances. This explains a common statement from lab analysts that "my GGA is failing high, but my blanks are fine".

5.2.14 Explain the potential reasons why GGA results could be unacceptably low.

LOW BIAS IN GGA
(1) Not enough seed
The main cause of low GGAs is either not enough seed material ("bugs") or a very weak seed material. Adjust the amount used until you consistently achieve GGA results in the acceptable range.

(2) GGA too old or contaminated
If GGA is too old, or has been broken down by contamination, low results will be observed. Discard expired or contaminated solutions.

(3) Seed materials too weak or variable

Try another GGA source. There are several different types/vendors that offer synthetic seeds. On occasion, there have been reports of poor quality lots.

5.2.15 How might you know if toxicity is occurring in the BOD test.

In order to ensure detection of sample toxicity, one must have prepared a sufficient number of sample dilutions and the individual dilutions must consist of significant differences in volume of the original sample. One other consideration is the degree to which BOD concentrations in individual dilutions differ. There must be a distinct trend in the data for the sample to be designated "toxic" and reported as such on the DMR.

The first symptom of sample toxicity is evidenced by a decrease in BOD concentration as sample volume increases. What this really means is that we're looking for a trend, and a trend realistically requires more than two data points. If only two dilutions are used, and the dilution with greater sample volume yields a lower BOD result, it COULD merely be a function of sample homogeneity. Having an additional dilution which confirms the initial two dilutions serves as a referee. Therefore, at least three (3) dilutions are necessary to effectively detect sample toxicity. These results do not mean a toxic sample because the range of the data is well within the precision capability of the test itself.

The next concern is that individual sample dilutions be sufficiently different to be able to detect a trend. For example, if dilutions of 100 mLs and 125 mLs are used, normal variability associated with the BOD test alone may make it difficult to discern any differences in BOD related solely to sample volume used.

The final consideration is to carefully evaluate the magnitude of difference between individual results. For example, consider the following data:

- a BOD of 6 for a 300 mL sample volume
- a BOD of 7 for a 200 mL sample volume
- a BOD of 8 for a 100 mL sample volume

Certainly it is true that these results “slide” downward with increasing sample volume. Before jumping to the conclusion that this is a “toxic” sample, one has to remember that BOD is a bioassay rather than a test which adheres to the more strict laws of chemistry. As a bioassay, BOD is not an exact science. In fact, Standard Methods suggests GGA control limits that represent only the mean ± a single standard deviation.

Given the expected accuracy of the BOD test and the close proximity of all three results to the theoretical LOD, it would be difficult to make the case that this is a toxic sample. It could be just coincidence that the results all “slide” in the same direction.

On the other hand, if a lab obtained the following results:

- over-depletion for a 50 mL sample volume
- a BOD of 85 for a 25 mL sample volume
- a BOD of 180 for a 10 mL sample volume

These results strongly suggest a toxic sample. The results are well above the reporting limit and there is a clear trend. Even the over-depleted 50 mL dilution could represent a BOD result as low as 45 mg/L.

5.2.16 What factors may cause toxicity in the BOD test?

Toxicity is the term used to define the conditions which would result in an apparent decrease in BOD concentration as the volume of sample increases. The phenomenon is frequently referred to as "sliding BOD" in reference to the typical observation that BOD "slides" down as sample volume used for analysis increases.

Sample toxicity can be caused by virtually anything that would adversely effect the health of the sample microbial population (which is required to utilize oxygen during the process of waste decomposition). Some things that would cause a toxic effect include, concentrations of heavy metals, sample pH extremes, concentrations of various inorganic (e.g., cyanides) and organic (e.g., pesticides) parameters.

5.2.17 Discuss reporting procedure if toxicity is suspected.

If there appears to be a sliding BOD (lower BOD with increasing sample size), there is a potential problem with sample toxicity. The best measure for BOD in this case would probably be the value obtained from the lowest acceptable sample size.

Toxicity can be insidious if only a single dilution meets depletion acceptance criteria. This is because operators have become programmed not to consider any dilution results for which the depletion exceed method-specified criteria. A toxic sample could look very much like the example below.

Sample Volume (mLs)	IDO (mg/L)	FDO (mg/L)	Depletion (mg/L)	BOD (mg/L)
50	8.49	0.10	>8.39	-----
100	8.40	2.40	6.00	18
200	8.31	5.19	3.12	5
		Average=		11.3

So … what we have is two dilutions--one with a BOD of 5 and the other with a BOD of 18. While this isn't the best precision in the world, many operators might be inclined to stop here and report the average of the two dilutions (11).

Ultimately, however, now is the time to at least evaluate the other data we have and see what it tells us. If we look at the dilution that over-depleted (see below) we can see that -- if calculated assuming a final DO of 0.1 mg/L was acceptable -- the result would be at least 50 mg/L. Now, the THREE results: 5, 18, and 50 mg/L -- give us more confidence that reporting the average would be significantly biased low.

Sample

Volume (mLs)	IDO (mg/L)	FDO (mg/L)	Depletion (mg/L)	BOD (mg/L)
50	8.49	0.10	[8.39]	[50]
100	8.40	2.40	6.00	18
200	8.31	5.19	3.12	5

So...what do we report for this sample?
• DO NOT report the “average” of dilutions (11.3)
• DO NOT report the highest value (18)
• The most appropriate result is to report ">" plus the highest BOD determined from any of the dilutions (i.e., “> 18”)
Furthermore, you MUST qualify these results as exhibiting "toxicity".

5.2.18 Discuss the problems associated with over-dechlorinating a sample.

Sodium sulfite is used to dechlorinate for BOD, because sodium thiosulfate has a significant oxygen demand if any excess is present. Because it is important to add only as much sodium sulfite as you need for dechlorination and no more, the operator must first determine how much chlorine is present before dechlorination. The excess could deplete DO and interfere with the test.

The most common dechlorinating agent is sulfite. The following forms of the compound are commonly used and yield sulfite (SO2) when dissolved in water. The greater the amount required to neutralize a standard concentration of chlorine, the greater the oxygen depletion affect.

Dechlorination Chemical	Theoretical mg/L Required to Neutralize 1 mg/L Cl2
Sodium thiosulfate (solution)	0.56 mg/L
Sodium sulfite (tablet)	1.78
Sulfur dioxide (gas)	0.9
Sodium meta bisulfite (solution)	1.34
Sodium bisulfite (solution)	1.46

Theoretical values may be used for initial approximations, to size feed equipment with the consideration that under good mixing conditions 10% excess dechlorinating chemical is required above theoretical values. Excess sulfur dioxide may consume oxygen at a maximum of 1.0 mg dissolved oxygen for every 4 mg SO2.

NOTE: Standard Methods specifies that sodium sulfite be used for dechlorination of BOD samples.

Section 5.3 - Total Suspended Solids (TSS)

5.3.1 Discuss the importance of TSS in wastewater analyses.

Total suspended solids (TSS) are those which are visible and in suspension in the water.

They are the solids which can be removed from wastewater by physical or mechanical means such as sedimentation, flocculation, or filtration. TSS will include the larger floating particles and consist of silt, grit, clay, fecal solids, paper, fibers, particles of food, garbage, and similar materials. Suspended solids are approximately 70% organic and 30% inorganic. TSS determinations may be used to assess wastewater strength, process efficiency, and loadings.

By reducing the TSS in your effluent discharge, you are going to get better disinfection, which will reduce your fecal coliform and/or E. coli counts, allowing you to maintain compliance.

The organic fraction of suspended solids is comprised generally of animal or vegetable matter, but may also include synthetic organic compounds. Organic compounds are substances which contain carbon, hydrogen, and oxygen, some of which may be combined with nitrogen, sulfur, and phosphorus. The principle organic compounds found in wastewater are proteins, carbohydrates, and fats, together with their products of decomposition. In general, they are combustible.

The inorganic fraction of suspended solids is inert and typically not subject to decay. Exceptions to this characteristic are certain mineral salts, such as sulfates, which, under certain conditions, can be broken down. Inorganic solids are frequently called mineral substances and include sand, gravel, and silt, as well as the mineral salts in the water supply which produce the hardness and mineral content of the water. In general, they are not combustible.

High TSS can block light from reaching aquatic vegetation. Photosynthesis is inhibited as the amount of light passing through the water is cut down. Without photosynthesis, aquatic plants produce less oxygen, which is a significant source of DO. If light is completely blocked from bottom dwelling plants, the plants will stop producing oxygen and will die. As the plants are decomposed, bacteria will consume what oxygen (DO) is present in the water. Low DO is a major contributor to fish kills.

High concentrations of TSS can also result in an increase in surface water temperature, because the suspended particles absorb heat from sunlight. Higher temperatures consequently result in a reduced ability of the water to hold DO.

5.3.2 Discuss what effect a low or high oven temperature will have on TSS test results.

A low temperature will not evaporate all the water, so the sample results will be higher. A high oven temperature will cause lower results, because of loss of organic matter by volatilization, loss of occluded water and water of crystallization, by driving off CO2, and causing conversion of bicarbonate. These changes occur to varying degrees over a wide range of temperatures.

5.3.3 Discuss the effects of oil & grease on TSS analysis.

Historically, Standard Methods included a statement that, "Results for samples high in oil and grease may be questionable because of the difficulty of drying to a constant weight in a

reasonable time." Under the interferences sections referenced in currently approved editions of Standard Methods (2540D), however, there is only the caution that, "Residues dried at 103 to 105°C may retain not only water of crystallization but also some mechanically occluded water."

In order to understand how to deal with this caution, we need to understand what is meant by "mechanically occluded" water. By "mechanical", what is meant is through physical forces, such as friction, abrasion, or some other physical-chemical interaction. The word "occluded" means to close up or block off. Thus it becomes clearer that the concern is in regards to water that is some how "blocked off" as a result of some chemical or physical interaction.

How does water become occluded during TSS analysis? Wastewater samples frequently contain surfactants as well as oils and greases. When surfactants encounter both oils and water, emulsifying colloids can be formed. Chemically, surfactants have both polar and non polar parts within a single molecule. Subsequently, a surfactant molecule has both hydrophilic (water-loving) and hydrophobic (water-hating) characteristics. When a surfactant's hydrophilic (polar) property is stronger than the hydrophobic (non-polar) property, it can form water occluded oil colloids. This essentially results in suspended "solids" particles that are composed of water trapped beneath a coating of oil, or "occluded water".

Because removal of occluded water is marginal at the method specified temperature (103-105 °C), drying samples to constant weight will be more difficult in samples with high levels of oils or greases. A longer drying time is required to remove all occluded water. This effect, of course, will be more pronounced the shorter the time that TSS filters are dried in the oven. Therefore those labs that employ a minimum one-hour drying time will be more impacted by samples with high levels of oil and grease.

Section 5.4 - Ammonia Nitrogen (NH3-N)

5.4.1 Discuss the requirement to distill samples prior to performing ammonia determinations.

When ammonia gas is dissolved in water, it will react with the water to form some ammonium ions. Depending upon the pH of the solution, the ratio of ammonia to ammonium will vary. At a higher pH, there is more ammonia. At a lower pH, there is more ammonium. In the distillation procedure, the sample pH is raised to 9.5 and the ammonia gas formed is removed by distillation. The ammonia gas is then absorbed in an acid solution where it is converted back to ammonium. The distillation removes the ammonia from the sample and leaves substances which may interfere with the analysis behind.

Interferences such as glycine, urea, glutamic acid, cyanates, and acetamide hydrolyze very slowly in solution on standing but, of these, only urea and cyanates will hydrolyze on distillation at pH of 9.5. If ammonia is determined using an ion specific ammonia electrode, the ammonia electrode uses a hydrophobic gas permeable membrane to separate sample solution from the internal solution, which is sensed by a pH electrode. Additional interferences include volatile amines and mercury, which forms strong complexes with ammonia. Note that residual chlorine reacts with ammonia and it must be removed by

sample pretreatment. If a sample is likely to contain residual chlorine, immediately upon collection, treat with a dechlorinating agent. Color and turbidity have no effect on the measurement, therefore distillation may not be necessary.

The Wisconsin State Laboratory of Hygiene & WDNR conducted a study of municipal wastewater effluents using the ion selective electrode. A statistical comparison of results with and without distillation was performed with the conclusion that distillation was not required. Consequently, the State Lab of Hygiene was granted a variance which extends to WWTPs. This variance is limited to:
• analysis using the ion-selective electrode.
• municipal effluents

If a facility is analyzing industrial or pre-treatment wastes, dairy or paper mill effluents (unique analytical matrix), or it is dealing with anything other than municipal wastewater, or using anything other than ISE, distillation is required.

Alternatively, NR 219 specifies the following:
"Manual distillation is not required if comparability data on representative effluent samples are on file to show that this preliminary distillation step is not necessary: however, manual distillation will be required to resolve any controversies".

5.4.2 Explain the operating theory of an ammonia electrode.

A "gas-sensing" type electrode is used for ammonia analysis. The three key parts of the ammonia electrode are an internal pH electrode (complete with reference electrode), a hydrophobic (impermeable to water) gas permeable membrane, and an electrolyte solution that fills the minute gap between the membrane and the pH bulb.

As ammonia, in gaseous form, diffuses across the gas-permeable membrane, a reaction takes place between ammonia and the water in which the electrolytes are dissolved. This reaction causes a change in the pH of the internal electrolyte solution which is in turn sensed by the pH electrode. The change in potential (measured as millivolts) is proportional to the concentration of ammonia in the sample.

Whereas most calibration relationships between analyte and instrument response are linear, it is important to note that the relationship between ammonia concentration and millivolts (electrode potential) is logarithmic. This explains why plots of ammonia calibrations use semi-logarithmic paper. In order to obtain a linear calibration, the log of ammonia concentration must be plotted against millivolt response.

5.4.3 Explain why temperature is the only variable that affects electrode response and why each ten-fold change in concentration should result in a slope (net difference in millivolt response) of -54 to -60 millivolts.

Response of any electrode is governed by physics. The slope of electrode response between concentrations of standards that are exactly ten-fold different from one another is governed by the Nernst factor. The Nernst factor (2.3 RT/nF), includes the Gas Law constant (R= 8.31451 Joules-Kelvin/mole), Faraday's constant (F=96.487 coulombs), the

temperature in °K (T) and the charge of the ion (n). For ammonia, n = 1 because the charge of the ion is "+1", or one hydrogen equivalent. Since "R" and "F" are constants, and "n" becomes a constant for any specific parameter, the factor and therefore electrode behavior is dependent only on temperature.

Substituting 298 for "T" (25 °C expressed as Kelvin), the Nernst factor calculates to be 59.16. This represents the theoretical electrode slope (millivolts per ten-fold increase in concentration) at 25 degrees C, the base temperature for electrode analysis.

A Nernst factor (slope) of 54 millivolts is associated with a temperature of 0°C, and a slope of 60 mV translates to 30 degrees C. Therefore, one would only expect a slope of 60 or more if the temperature of the calibration solution was 30°C. At 20°C, the theoretical slope is 58.15 mV.

Many ion meters display the slope as a percentage of the theoretical value. For example, a 98.5% slope is equivalent to a slope of 58.27 mV (at 20 degrees C).

5.4.4 Discuss how to troubleshoot blank problems in the ammonia test.

Ammonia-free water should be used to prepare all reagents and standards. Ammonia-free water can be prepared by either ion-exchange or distillation.

- Ion exchange: passes reagent water through an ion-exchange column containing a strongly acidic cation-exchange resin mixed with a strongly basic anion-exchange resin. If blanks are still high, use a strongly acidic cation-exchange resin alone.
- Distillation: Re-distill after adding 0.1 mL concentrated sulfuric acid (H_2SO_4) per L of distilled water.

The presence of ammonia gas in the air makes it difficult to store ammonia-free water in the laboratory. To store ammonia-free water properly, store in a sealed glass container. Add about 10 grams of a strong acidic cation-exchange ion-exchange resin. Allow resin to settle. Decant ammonia-free water. If blanks are still high, replace the resin or prepare fresh ammonia-free water.

5.4.5 Explain why calibration standards and samples must be at the same temperature when using the ion-selective electrode.

Ammonia electrodes function according to the physical constraints of the Nernst equation, and temperature is the only variable. Each one degree (°C) change in temperature is associated with a 1-2% error due to changes in the electrode slope.

5.4.6 Discuss why, although an approved technique, the Nessler method is not a good choice for ammonia determinations.

The Nessler technique has concentration range limitations; this technique is only appropriate for levels of 0.05 to 1.0 ppm. More important, however, is the concern over the toxicity of the Nessler reagent itself, both to the environment and the health risks it poses for analysts. Nessler reagent contains 100 grams of mercuric iodide (HgI_2) per liter. The Nessler technique calls for the addition of 2 mL Nessler reagent to 50 mL of sample in a

Nessler tube. Based on the percent composition of mercury in the Nessler reagent, each 2 mL of Nessler reagent contains 88 mg of pure mercury, which is added to 50 mLs of sample.

Doing a concentration calculation, 88 mg of mercury in a total volume of 52 mLs (50 mLs sample + 2 mLs Nessler reagent) results in a mercury concentration of 1.69 mg/L in each sample. For comparison purposes, the limit in drinking water is 0.0002 mg/L. That means that 50 mLs of sample plus 2 mLs of Nessler reagent contains nearly 10,000 times the maximum allowable concentration of mercury allowable in drinking water.

Disposal becomes an issue because the contents of just ONE Nessler tube would have to be diluted with about 130 gallons of reagent water to make it safe to drink. Consequently, dumping samples down the sink after analysis is not an option, as the mercury will just end up in the sludge, making landspreading more problematic. All Nessler samples should be treated as hazardous waste and disposed of accordingly.

5.4.7 Discuss the importance of pH and temperature in determining ammonia toxicity.

Ammonia-nitrogen is a major end product of fish metabolism, it is toxic to aquatic life and toxicity is affected by system pH. Ammonia-nitrogen (NH3-N) has a more toxic form at high pH [un-ionized ammonia or NH3] and a less toxic form at low pH [ionized ammonia or NH4+]. In addition, ammonia toxicity increases as temperature rises. The un-ionized form is considered more toxic since it can diffuse passively across the gill membrane.

At or below a pH of 8.5, less than 20% of ammonia exists in the highly toxic un-ionized (gaseous) form. Above a pH of 8.5, the percentage of ammonia that exists in the un-ionized form increases rapidly.

The effect of temperature increases is far less significant than increases of pH. Consider a lagoon or pond system containing 10 mg/L ammonia (NH3-N). As can be seen from the table in Figure 5.4.07, the temperature change during the day from 20°C to 30°C accounts for less than 20% of the increase in toxicity as compared to a rise in pH from 7.0 to 8.0.

Essentially, the higher the pH and temperature, the greater the proportion of total ammonia in the system will be in the form of the highly toxic un-ionized ammonia (NH3). A useful rule of thumb is that at a pH of 8 ammonia is 10 times more toxic than at a pH of 7, and at 20°C it is two (2) times more toxic than at 10°C.

To calculate the amount of un-ionized ammonia present, the Total Ammonia Nitrogen (TAN) must be multiplied by the appropriate factor selected from the table in Figure 5.4.07 using the pH and temperature from your water sample.

Figure 5.4.07

pH	Temperature													
	42.0 (°F)	46.4	50.0	53.6	57.2	60.8	64.4	68.0	71.6	75.2	78.8	82.4	86.0	89.6
	6 (°C)	8	10	12	14	16	18	20	22	24	26	28	30	32
7.0	.0013	.0016	.0018	.0022	.0025	.0029	.0034	.0039	.0046	.0052	.0060	.0069	.0080	.0093
7.2	.0021	.0025	.0029	.0034	.0040	.0046	.0054	.0062	.0072	.0083	.0096	.0110	.0126	.0150
7.4	.0034	.0040	.0046	.0054	.0063	.0073	.0085	.0098	.0114	.0131	.0150	.0173	.0198	.0236
7.6	.0053	.0063	.0073	.0086	.0100	.0116	.0134	.0155	.0179	.0206	.0236	.0271	.0310	.0369
7.8	.0084	.0099	.0116	.0135	.0157	.0182	.0211	.0244	.0281	.0322	.0370	.0423	.0482	.0572
8.0	.0133	.0156	.0182	.0212	.0247	.0286	.0330	.0381	.0438	.0502	.0574	.0654	.0743	.0877
8.2	.0210	.0245	.0286	.0332	.0385	.0445	.0514	.0590	.0676	.0772	.0880	.0998	.1129	.1322
8.4	.0328	.0383	.0445	.0517	.0597	.0688	.0790	.0904	.1031	.1171	.1326	.1495	.1678	.1948
8.6	.0510	.0593	.0688	.0795	.0914	.1048	.1197	.1361	.1541	.1737	.1950	.2178	.2422	.2768
8.8	.0785	.0909	.1048	.1204	.1376	.1566	.1773	.1998	.2241	.2500	.2774	.3062	.3362	.3776
9.0	.1190	.1368	.1565	.1782	.2018	.2273	.2546	.2836	.3140	.3456	.3783	.4116	.4453	.4902
9.2	.1763	.2008	.2273	.2558	.2861	.3180	.3512	.3855	.4204	.4557	.4909	.5258	.5599	.6038
9.4	.2533	.2847	.3180	.3526	.3884	.4249	.4618	.4985	.5348	.5702	.6045	.6373	.6685	.7072
9.6	.3496	.3868	.4249	.4633	.5016	.5394	.5762	.6117	.6456	.6777	.7078	.7358	.7617	.7929
9.8	.4600	.5000	.5394	.5778	.6147	.6499	.6831	.7140	.7428	.7692	.7933	.8153	.8351	.8585
10.0	.5745	.6131	.6498	.6844	.7166	.7463	.7735	.7983	.8207	.8408	.8588	.8749	.8892	.9058
10.2	.6815	.7152	.7463	.7746	.8003	.8234	.8441	.8625	.8788	.8933	.9060	.9173	.9271	.9389

5.4.8 Discuss the procedure required to determine whether an ammonia probe is malfunctioning.

PERFORMING AN INNER-BODY CHECK

1. Pour about 50-100 mL of pH 4.00 buffer into a beaker and 50-100 mL of pH 7.00 buffer into another beaker. Label each beaker.
2. Disassemble your ammonia probe.
3. Once you have disassembled the probe, be careful with it because the glass at the bottom of the inner body is very fragile. If you break it, you WILL be buying a new probe.
4. Make sure the probe is connected to the meter and put the meter into the millivolt (mV) mode.
5. Place the beaker containing the pH 7 buffer with NaCl on a stir plate, add a 1" stir bar and stir at slow to medium speed.
6. Clamp the inner body into a probe holder and immerse the probe in the beaker on the stir plate, being careful to NOT lower it so far that it is hit by the stir bar.
7. The reading should stabilize within two minutes. A “stable” reading is a reading which drifts 0.1 mV/minute or less. Record this reading paying attention to the minus sign (-), if present.
8. Remove the inner body from the solution, rinse it with DI water, and repeat steps 5-7 above using the pH 4.00 buffer with NaCl.
9. From the two mV readings obtained, calculate the difference in millivolts. Remember to take any minus signs into account. For example, if the reading in the 7 buffer was -25 mV, and the reading if the 4 buffer was 145 mV, the difference is 160 mV.

The ideal difference is 175 mV. Normally any difference over 150 mV is satisfactory. Usually, if the sensor is bad, the difference will be 2 mV or less, so it should be pretty obvious whether the sensor is good or not. If the probe fails the inner body check, there is NOTHING you can do to make it work and it will have to be replaced.

If the probe passes the inner body check, chances are good that the problem lies elsewhere: bad membranes, bad filling solution or improper lab technique.

5.4.9 Discuss the linearity of ammonia electrodes.

Most ion-selective electrodes are actually non-linear over a portion of the lower region of a calibration range. In the linear regions of the curve, only two standards are needed to determine a calibration curve (although NR 149 requires at least 3 calibration levels). In non-linear regions, at least three calibration standards must be used. Most laboratories use 0.2 mg/L as their low concentration calibration standard, which falls within the non-linear region of the probe, particularly as probes become heavily used.

Vendor representatives of newer “Orion” meters strongly encourage users to use the on-board software to calculate ammonia concentration when calibration is based on three or more standards. That is because when more than two standards are used for calibration, the instrument software relies on a specialized function that is based on the Nernst equation, fully reproducible, and which accounts for non-linear response from the probe.

The use of the non-linear function requires a conceptual change in generally acknowledged acceptance criteria for a calibration (see Figure 5.4.09A). Referenced methods all state that the acceptable slope for an ammonia ISE analysis is 54 - 60 millivolts (mV). Because of the non-linearity, and the mechanism used to derive the non-linear Nernst function, it is possible for the slope of a calibration to exceed 60 mV and still be acceptable. In no case, however, would a calibration having a slope of less than 54 mV be acceptable.

The calibration function operates by solving the Nernst equation with one additional variable, called the “blank”, or "auto-blank". The “blank” referred to is not just for a background blank. It may be a true reagent blank, it may indicate traces of the analyte ion in the reagents, or it may be the “mud” value of the electrode.

Alternatively, it may be an interferent in the reagents that becomes apparent at low ammonia levels. Finally, it could be some combination of all of these things. Basically, the value is only serving as a correction factor to the calibration point in the log function. It should not ever be reported as a blank, nor should it be used for a “blank correction” of results on a lab report. It can be, however, an important tool for use in evaluating a given calibration.

Figure 5.4.09B shows four (4) calibration curves and the resulting calibration parameters for each. Note that for the calibration labeled ”Poor”, the electrode slope still meets method requirements. The value of the non-linearity function

coefficient termed “Blank”, however, exceeds the concentration of the lowest calibration standard (0.2 ppm). Consequently data suggest that in addition to evaluating the electrode slope, users should also consider the “blank” concentration relative to that of the lowest calibration standard. It could be argued that the quality of the calibration decreases as the value of the “blank” term increases.

Figure 5.4.09A

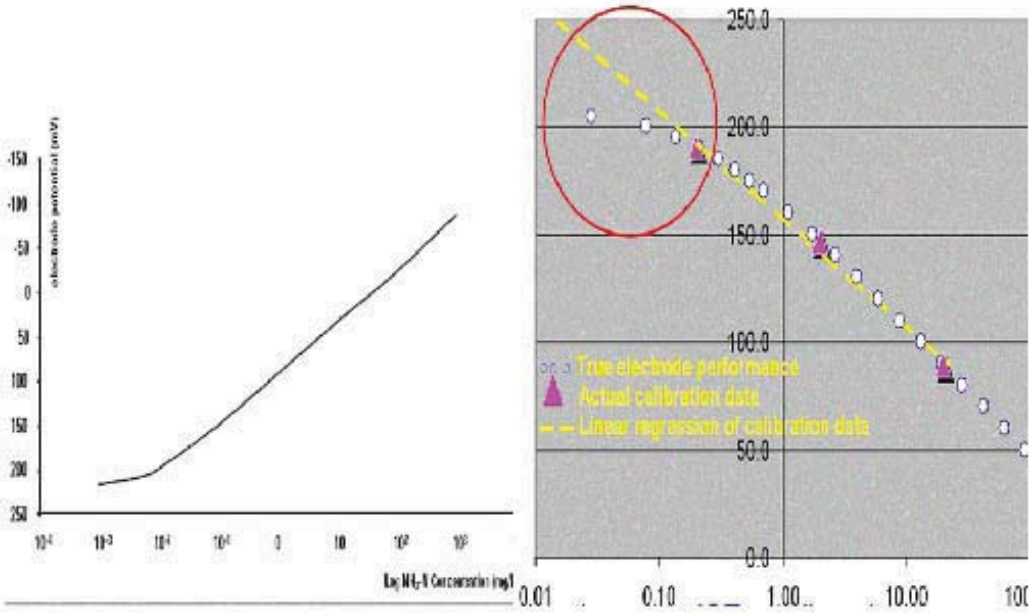
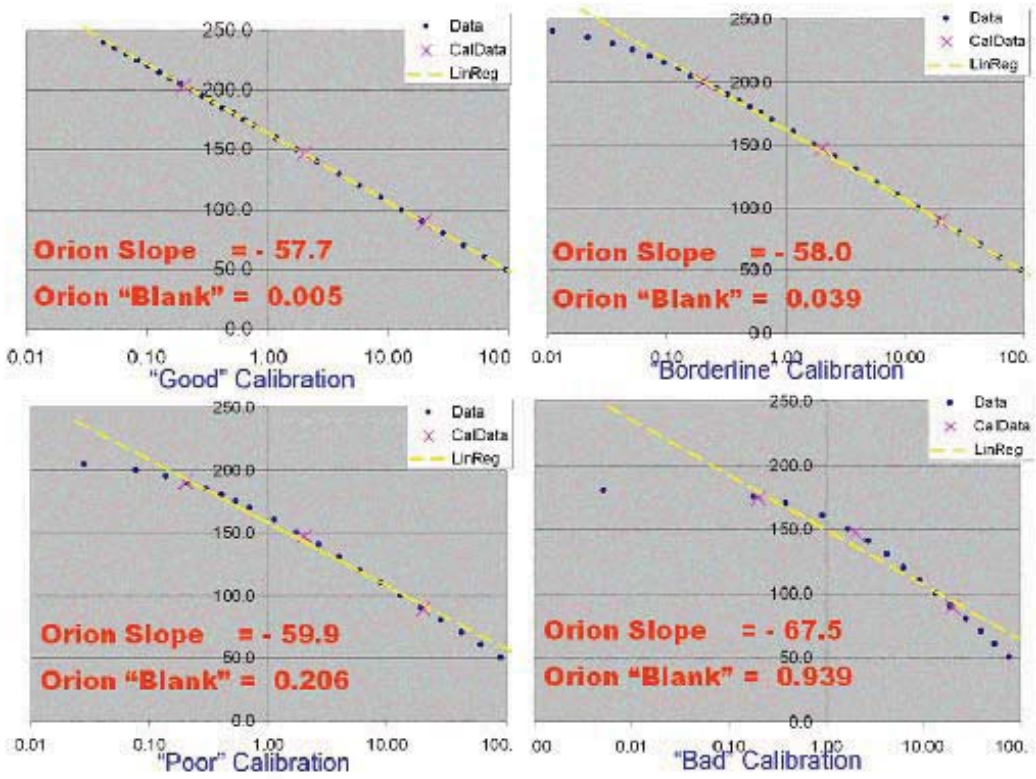


Illustration from: *User Guide: High Performance Ammonia Ion Selective Electrode, 2007 Thermo Fisher Scientific Inc.*

Ammonia ISE non-linearity at low concentrations

Figure 5.4.09B



5.4.10 Discuss criteria for valid Algorithm

Thermo-Fisher Scientific (Orion), in response to concerns that because the probe algorithm allows for a depressed slope on the lower segment, a lab could conceivably incorrectly make up its 0.2 ppm cal standard but correctly prepared 2 and 20 ppm standards, identified some key calibration criteria. In their testing, Thermo found that the "accident" or "errors" in preparation of standards caused the slope for segment 1 to be less than 40 mV, thereby violating criteria 1.

Thermo's engineers came up with four (4) criteria that must be met in order for the algorithm to be valid. The idea was for the criteria to consider the TWO segment slopes, rather than simply the overall slope of both segments. The criteria apply to all monovalent ISE probes (NH3, NO3, F-).

1. Slope 1 is the low to midpoint segment; slope 2 is the mid to upper point segment. The delta mV of slope 1 MUST be -40 to -60 mV. The reason for this allowance is that the electrode is potentially working in the non-linear region so we need to deviate from the linear expectation.

2. Slope 2 MUST be within -54 to -60 mV

3. Slope 1 MUST be greater than (less negative than) Slope 2. [Slope 2 must basically have a higher delta mV (change in) per decade than slope 1] For

instance, a calibration with a slope 1 of -60 mV and then a slope 2 of -58 mV would fail.

4. This is less of a criterion and more of a definition. When Criteria 1, 2, and 3 are met, and you plug the 3 point calibration into the meter, the resultant meter slope WILL be -54 to -65 mV.

A second source calibration verification standard at a level of about 25 to 50% of the midpoint standard (0.5 to 1 ppm) is strongly encouraged.

Section 5.5 - Total Phosphorus (TP)

5.5.1 Discuss the calibration blank required for phosphorus testing.

Blanks are used to assess the degree of contamination attributed to the measurement process. There are many types of blanks, each of which serves a different purpose. Not all steps of the analytical process can be directly evaluated from a single type of blank. However, by subtracting the response (absorbance) of one type of blank from another, we can indirectly identify the level of contamination associated with any one step of the analytical process. Both calibration blank and method blank are required for phosphorus testing.

CALIBRATION BLANK
A calibration standard containing no added analyte, but all the other reagents that are in other calibration standards, such as color reagent, and digestion reagent (if standards are digested). In many cases, the calibration blank may be virtually identical to a method blank.

Generally, calibration blanks:
* Consists of the solvent used plus all of the same reagents used to prepare the calibration standards.
* If the standards are digested - the same as the samples are, then the calibration blank consists of reagent water plus all other reagents including the combined color reagent.
* If the standards are not digested, therefore handled differently than the samples, then the calibration blank consists of reagent water plus combined color reagent, but not the digestion reagents.
* Indicates the absorbance response of a zero concentration standard (0.0 mg/L). This blank is not used to zero the instrument. The absorbance of this blank is measured and used in the calibration curve as (x = concentration = zero; y = response = measured absorbance). It is possible for the measured absorbance of this blank to be zero, but it is not expected to be zero.

5.5.2 Discuss the METHOD and SAMPLE COLOR types of blanks required for phosphorus testing.

METHOD BLANK

The purpose of a method blank is to provide a measure of contamination from sample preparation through testing. A method blank is carried through all the steps of sample preparation and analysis as if it were an actual sample. The same solvents and reagents that are used with the actual samples are added to a volume of lab reagent water

proportional to a typical sample volume. The method blank accounts for contamination that may occur during sample preparation and analysis. This contamination could come from reagents, glassware, or even the laboratory environment. If calibration standards are processed (digested) then the calibration blank and method blank are essentially the same.

While the method blank quantifies the TOTAL response or contamination resulting from the whole preparation and analytical process, by itself the method blank cannot be used to determine at which step(s) any contamination identified occurred.

If you use a single blank to zero the spectrophotometer, serve as a calibration blank (or 'zero') standard, and to serve as a method blank, the result will always be effectively zero. This process amounts to blank subtraction which is not an allowable practice for most analyses. In addition, this approach can mask gross contamination in the laboratory. For example, if the method blank, standards and samples are all blue, the laboratory clearly has a significant background contamination problem. However, if the spectrophotometer is zeroed with the "blue" method blank, there will be no indication there is a contamination problem. Despite any dark blue color, the method blank result will be below the detection limit. Furthermore, zeroing with the method blank does not provide a suitable method blank according to laboratory certification rules.

SAMPLE COLOR BLANK

This kind of blank is not routinely measured; it is used more in unique situations or for troubleshooting purposes.

A sample color blank (sometimes known as a 'color blank') is prepared by adding all of the reagents except the one(s) directly involved in producing the color reaction to a sample of the water to be analyzed. This is logical, because once the characteristic color is produced, then we can only measure the combined color associated with the sample, the reagents, and the preparation and analytical procedures. The reason for this blank is that "apparent color" may be produced by suspended material in the water. Inclusion of this absorbance with the instrument response can be misleading and will indicate higher amounts of the target analyte than are actually present.

A sample color blank result is obtained by determining absorbance on an aliquot of sample to which the COLOR REAGENT is added and subtracting from that determination the results of a second aliquot of sample to which the 'SAMPLE COLOR BLANK REAGENT' solution is added. For total phosphorus analysis, sample color blank reagent is prepared by substituting lab reagent water for the potassium antimonyl tartrate and the ascorbic acid.

5.5.3 Discuss additional blanks which may be useful in troubleshooting phosphorus testing.

Additional blanks that can be useful in troubleshooting include the following:

INSTRUMENT BLANK

An instrument blank is used to establish a baseline for how much light passes through the sample compartment without a sample being present. In addition to absorbance stemming

from the solvent itself, any reduction in light transmission that is caused by a film or scratches on the cuvette surface are also taken into consideration. In wastewater lab testing, the solvent is lab reagent water.

Therefore, the instrument blank:
- Consists of only the solvent used - which is reagent water for total phosphorus.
- Allows instrument to be zeroed on background attributed to just the solvent used and therefore what the "Zero" should be when using the solvent.

REAGENT BLANK
This kind of blank is not routinely measured; it is used more in unique situations or for troubleshooting purposes. One of the more difficult blanks to prepare, the reagent blank, is designed to quantify the amount of background response (or contamination) that results from absorbance at the characteristic wavelength by the reagents themselves. The difficulty is in adding only those reagents that are not directly involved in developing the characteristic color associated with the analyte in a colorimetric procedure.

For example, the phosphorus color reagent is composed of four different reagents, several of which can have "color" and therefore may absorb light at the characteristic wavelength. In order to absolutely quantify the contribution of each of the four, separate blanks would have to be prepared by making four separate color blank solutions, each with one of the four reagents. Lab reagent water would be substituted at the same proportions for the other three reagents.

For total phosphorus analysis, a commonly used reagent blank for the color reagent solution can be prepared by substituting lab reagent water for the potassium antimonyl tartrate and the ascorbic acid.

Refer to the term "sample color blank" for a close relative of the reagent blank. With a sample color blank, we are trying to quantify the background response associated with the sample itself. Typically, in total phosphorus analysis, the digestion process destroys any natural sample "color". If a lab were to test for orthophosphate however, a sample color blank - for each sample - is frequently required.

5.5.4 Explain how the natural color of a sample can affect phosphorus analysis and how to correct for this problem.

Some plants develop seasonal color due to algae, etc. This color (or turbidity) may register background absorbance ...which amounts to high bias on phosphorus analysis.

Optimally, subtract "background" from true sample absorbance. This requires the measurement of absorbance from a "sample color blank".

COMBINED COLOR REAGENT (100 mLs):
50 mL 5N sulfuric acid
5 mL potassium antimonyl tartrate

15 mL ammonium molybdate
30 mL ascorbic acid

SAMPLE COLOR BLANK REAGENT (100 mLs):
35 mL reagent water
50 mL 5N sulfuric acid
15 mL ammonium molybdate

Sample Absorbance = Absorbance of [sample + color reagent] - Absorbance of [sample + sample color BLANK reagent]

5.5.5 Discuss the causes and solutions for reduced absorbances in the phosphorus test.

Excess potassium persulfate present after digestion can cause weak color development. Make sure you are using the proper amount of persulfate.

Weak or reduced absorbances could also be an indication of instrument problems related to the wavelength.

Finally if reduced response (from typical readings) is observed only in standards or QC samples prepared from standards, it may be an indication that the stock standard used to prepare the standard was prepared incorrectly. A "second source" standard can be used to compare responses.

5.5.6 Explain what might be the cause of slow color development in samples or standards.

When potassium antimonyl tartrate is absent, old, or weak, the color reaction proceeds slowly.

5.5.7 Discuss what might cause mixed color reagent to be blue instead of clear or very pale yellow in color.

Phosphorus contamination can cause the color reagent to be blue in color. Alternately, if sulfuric acid is omitted or a weak acid solution is used to prepare the color reagent, the final color of the solution can be blue. Sulfuric acid solution used to prepare color reagent must be 5N and represent 50% of the total volume of the color reagent.

5.5.8 Explain what might be the causes and the solutions of rapidly fading blue color in samples or standards during the color reaction step for phosphorus testing.

Incorrect acid concentration or bad antimony solution will cause color to fade. Check acid strength; check post-digestion pH adjustment; prepare fresh color reagent.

5.5.9 Discuss the effects of exceeding color development time in the phosphorus analysis.

The referenced methods specify that after addition of color reagent, absorbance should be measured after at least 10 but not more than 30 minutes (between 2 and 8 minutes for HACH Test 'N Tube). The reaction continues to progress after these time limits, with absorbance continuing to increase over time. Consequently, allowing more than 30 minutes (8 minutes for Test 'N Tube) could result in results biased high.

Section 5.6 - Total Residual Chlorine (TRC)

5.6.1 Explain how the chlorine electrode works.

- The electrode is based on iodometric measurement of chlorine.
- Iodide (I-) and hydrochloric acid (H+) are added to a sample.
- Iodide reacts with chlorine to form iodine.
- The iodine concentration is equal to the chlorine concentration.
- The ISE contains a platinum sensing element and an iodine sensing reference element.
- The platinum element develops a potential that depends on the relative amount of iodine and iodide in solution.
- The iodine-sensing element develops a potential that depends on the iodide level in solution.
- The meter measures the difference between these potentials (which therefore provides the iodine concentration).
- Iodine concentration = total residual chlorine concentration.

5.6.2 Discuss the major differences between the ion selective electrode analyses of ammonia and chlorine residual.

The total residual chlorine ISE analysis differs from the ISE analysis of ammonia in that:

- The ammonia electrode measures the potential caused by ammonia GAS crossing a gas permeable membrane whereas chlorine electrodes measure potential caused by IONS crossing the membrane.
- The electrode slope for chlorine is positive (millivolts increase with increasing concentration). Ammonia has a negative slope (millivolts increase with decreasing concentration).
- The slope change in mV per decade of concentration is 29.0 for chlorine electrodes vs. 58.3 for ammonia at 20 °C.

5.6.3 Discuss appropriate calibration levels for chlorine by ISE.

In order to meet the goal of having a limit of quantitation of 0.1 ppm, the lowest calibration standard should be fixed at a concentration of 0.1 ppm. It is preferable to use at least five calibration levels, and the uppermost calibration standard concentration should not exceed 2.0 ppm.

Other critical considerations associated with calibration of residual chlorine by ion-selective electrode are:

- Use the more stable potassium iodate standard for calibration.
- Avoid calibrating below 0.1 ppm due to non-linearity.
- Check the slope from 0.2 to 2.0 (start above 0.1).
- Allow approximately 30-45 minutes for a 5-point calibration.
- Like other electrode method (ammonia), the ISE method is extremely temperature-sensitive.

Section 5.7 - Process Control

5.7.1 Discuss the calibration procedures for a pH meter.

pH Meter Calibration - Key Points

To avoid problems associated with having to have samples and buffers at the same temperature:

1. After receiving a new pH electrode, soak it for one day in pH 7 calibration solution in order to hydrate the bulb.

2. Connect the electrode(s) to meter. Use a automatic temperature compensating probe (ATC) along with the pH electrode (i.e., Orion Triodes).

3. Always use 2 point calibration. Choose either 4.01 and 7.00, or 7.00 and 10.01 buffers, whichever will bracket your expected sample range.

4. Rinse the electrode(s) and place into the 7.00 buffer.

5. Turn meter on and place in calibration mode.

6. When the meter response stabilizes, the temperature-corrected value for the buffer is displayed, enter the value.

7. Rinse the electrode(s) and gently blot the end dry with a paper towel. (Note: Do not wipe, just blot to prevent static electricity affecting the membrane.)

8. Place electrode into the second buffer. When the meter response stabilizes, the temperature-corrected value for the buffer is displayed, enter the value.

9. After the second buffer value has been entered, the electrode slope should be displayed. Record the slope. Slope should be between 92-102%. The slope is a percentage of the theoretical slope (59.12 mV/pH unit). For example, a 98.5% slope is equivalent to a slope of 58.27 mV/pH unit. If slope is above 102%, this may indicate contaminated buffers. A slope that is less than 92% may indicate a blocked junction and the electrode may need cleaning or should be replaced. Consult your electrode manual from the manufacturer for specific information about troubleshooting the probe.

10. Rinse the electrode(s) and place into the sample. Record pH and temperature directly from the meter display.

5.7.2 Discuss critical consideration associated with pH calibration.

- Never reuse buffer solutions. Each calibration event should start with fresh buffer solutions. A pH meter and electrode system should be recalibrated after 4 hours since the last calibration.

- The most common cause of error in pH measurements is temperature. The slope of a pH electrode is highly dependent on temperature, and pH buffer values and sample values change with temperature. For the most accurate results an ATC probe is always

recommended.

• Store your electrodes according to manufacturer's recommendations. If you will be measuring pH daily, the response time will be faster if the probe is stored in a pH 7.00 solution with a little KCl added.

5.7.3 Describe how to inspect, predict useful life, and store a pH electrode.

The reference junction for refillable electrodes should be clean and white in appearance. A discolored junction indicates plugging which will prevent proper solution seepage. Plastic gel filled probes have to be replaced periodically.

Difficulty in doing a two-buffer calibration and slow response times are two prime indicators of a need for probe rejuvenation (for refillable glass electrodes), or replacement (for gel-filled electrodes). A gel-filled probe may last longer if the probe is stored in the correct storage solution.

pH electrodes should not be allowed to dry due to neglect. For rapid response and longer life, electrodes should be stored in a solution recommended in the electrode manual. However, if a glass probe is to be stored for a long time, it could be dismantled, well cleaned, and stored dry.

5.7.4 Knowledge deleted.

5.7.5 Knowledge deleted.

5.7.6 Identify the possible causes and corrective actions for the common pH meter problems:

INSTRUMENT GIVES NO READING.
Some meters will not give any reading if there is a problem with the power source. Check all connections and the power source. After even a brief power outage you may need to disconnect and reconnect the meter from the power source.

VERIFICATION OF A 2-POINT CALIBRATION EXCEEDS 0.1 pH UNIT.
• Check the condition of both buffer solutions.
• Check the condition of the reference junction (for a standard probe). A plugged reference junction will cause slow results making calibration difficult. The rubber sleeve must not be over the hole. The solution level must be above the level of sample in the beaker. Cracks or scratches mean that the probe must be replaced.
• If a gel-filled probe is being used it may have passed its effective life (generally 4-6 months or more, depending on use).

SLOW INSTRUMENT RESPONSE TIME.
A slow response time is caused by:
• A plugged reference junction.
• Leaving the fill hole closed while running the test.

• A dirty probe.
• An expired gel-filled probe. A sample with very low ionic strength will also cause slow response.

THE NEEDLE WILL NOT STABILIZE (ANALOG METERS).
The problem could be:
• In the meter, shorting may indicate if there is a problem with the meter. Static electricity can cause problems with erratic action in an analog meter. Magnetic fields induced by other wiring close to the meter, especially if significant amps are drawn through that wiring.
• The connection of the probe to the meter.
• The probe itself.
• The needle or digital display will not stabilize if the connection between the probe and meter is not tight. Gently manipulate the connection wire near the meter and near the probe. Check for a reading change.

PLUGGED REFERENCE JUNCTION.
Always clean the probe immediately after use. Clean using the method suggested by the probe manufacturer. There are different solutions suggested, depending upon whether the blockage appears to be mineral or organic. Soak the probe in the solution, rinse well, and refill. If this does not work, replace the probe. A gel-filled probe could be used and replaced every 4-6 months. With a regular probe, schedule the probe rejuvenation procedure regularly. Some probes have a replaceable reference probe. Other probes are designed to be taken apart to clean the reference junction. One model pH unit has a positive displacement reference solution pump, where instead of having the solution seep through a porous material, it is pumped to the measurement site.

CRYSTAL FORMATION INSIDE THE PROBE.
Rinse the probe with very warm distilled water until crystals are dissolved, then refill with new probe solution.

5.7.7 Discuss a procedure that can be used to determine if the pH probe is operating correctly.

1. To test a pH electrode, place it in a fresh pH 7 buffer.
2. Press pH/mV to use the mV mode, and note the millivolt reading.
3. Repeat for either a pH 4 or pH 10 buffer.

The electrode signal must be within the limits shown below (when temperature is near 20°C).

Electrode Test	Criteria
pH 7	0 ± 15 mV
pH 4	174 ± 15 mV (159 to 189 mV)
pH 10	-174 ± 15 mV (-159 to -189 mV)

In a perfect world, ideal mV readings are 0 mV in 7 pH, +174 mV in 4 pH, -174 mV in 10 pH.

5.7.8 Discuss the advantages and disadvantages of various types of pH electrodes.

Glass bodied pH electrodes may be used in most sample types. Epoxy bodied pH electrodes are designed for rugged environments, multiple-user situations, and field or plant applications. Epoxy bodied pH electrodes should not be used in organic solvents.

The pH meters in most laboratories usually have convenient, compact, combination electrodes made by combining the reference and pH measuring electrodes into one housing. Combination electrodes work efficiently for most purposes but are not suitable for measuring the pH of certain "difficult" samples.

GLASS vs. EPOXY (PLASTIC) BODY ELECTRODES

Glass Body Electrodes:

- Slightly faster (due to larger surface area of bulb)
- Easier to Clean (larger bulb surface area)
- Available in more shapes for special applications
- Withstands higher temperatures (typically up to 100°C; plastic up to 80°C)

Epoxy (Plastic) Body Electrodes:

- More rugged (the electrode stem bulb is well protected)
- Costs less than glass electrodes

GEL-FILLED vs. REFILLABLE ELECTRODES

Gel-Filled:

- Requires less maintenance; no filling required
- Resists higher pressure (+15-30 psi)
- Will only last between 6 months to 18 months if properly stored
- Low initial cost

Refillable Electrodes:

- Electrode could last longer if electrolyte is changed
- A different electrolyte, for example, silver-free or calomel for special applications can be used.
- Slightly faster response due to greater flow of electrolyte into the sample
- Will last longer if properly maintained (2-3 + years)

Glass electrodes have two disadvantages:

1) Measuring solutions containing particulates can damage the glass membrane.
2) The glass membrane is easily broken.

5.7.9 How is pH affected by nitrification?

The optimum pH for nitrification is 7.5 to 8.5 s.u. As ammonia is converted to nitrite and nitrate, alkalinity decreases and pH of the wastewater may drop.

For every 1 pound of ammonia nitrogen oxidized, 7 pounds of alkalinity are used up. During

nitrification, the oxygen molecule is stripped from the alkalinity in the wastewater (as calcium carbonate, or CaCO3). As the CaCO3 is destroyed, the buffering action that it provides is also decreased, lowering the pH.

5.7.10 Explain why the fill-hole on a reference electrode should be open when testing for pH, and closed when the probe is not in use.

The reference solution should seep out of the reference junction during measurements. If the fill-hole is closed while using a probe, the reference solution will not seep out as needed, because vacuum will prevent it from doing so. The fill-hole should be closed when a pH probe is not in use to prevent the probe solution from seeping out of the reference junction and crystallizing on the probe and storage container.

5.7.11 Explain how total Kjeldahl Nitrogen (TKN) and Nitrate Plus Nitrite Nitrogen are measured and how they are used in process control:

TOTAL KJELDAHL NITROGEN (TKN)
TKN is the sum of the organically held nitrogen plus the ammonia nitrogen present in a sample. It does not include the oxidized nitrate plus nitrite nitrogen. It is measured by digesting the sample to break down all the organically held nitrogen and converting it into ammonia. The ammonia generated is then distilled from the sample into an acid solution. The ammonia concentration can be determined by several methods.

In order to remove nitrogen from a wastewater treatment facility you must understand the different forms of nitrogen and how they relate to each other.

Total Nitrogen (TN) is the sum of all nitrogen forms = TKN + NO2 + NO3
TKN = Total Kjeldahl Nitrogen and represents the sum of NH3 + Organic Nitrogen
NH3 = Ammonia Nitrogen
Organic Nitrogen is derived from amino acids & proteins (e.g., urea, uric acid)
NO2 = Nitrite
NO3 = Nitrate

Ammonia (NH3) values represent approximately 60% of the TKN of a waste, and the organic nitrogen is generally removed in the settled sludge. Also, TKN generally equals 15 - 20 % of the BOD of raw sewage.

NITRATE + NITRITE NITROGEN
Nitrate and nitrite nitrogen are most often tested by the cadmium reduction method. The electrode is not acceptable for wastewater effluent permit required testing. In addition, the electrode is specific only for nitrate, not nitrite. Results should be reported in mg/L as nitrogen (N).

The levels of nitrate and nitrite become important due to their involvement in denitrification and disinfection.

Nitrite levels should be very low throughout the entire treatment process. High levels of nitrite (NO2) in the system indicate there may be a problem with the nitrification cycle.

Nitrosomonas bacteria are harder to kill than Nitrobacter bacteria. If the Nitrobacter bacteria are killed off, the Nitrosomonas bacteria will continue working on the ammonia (NH3) and you will have a jammed cycle with high levels of nitrite (NO2). An effluent with high nitrite (NO2) concentrations will be difficult to disinfect because of the tremendous chlorine demand it poses.

Denitrification is an anaerobic process (meaning without oxygen) in which the oxygen bound in nitrate (NO3-) becomes the primary oxygen source for microorganisms. When bacteria break apart nitrate (NO3-) to gain the oxygen (O2), the nitrate is reduced to nitrous oxide (N2O), and nitrogen gas (N2). Since nitrogen gas has low water solubility, it tends to escape as gas bubbles. These gas bubbles can become bound in the settled sludge in clarifiers and cause the sludge to rise to the surface.

An advantage of denitrification is the production of alkalinity (which will help buffer against pH changes) and an increase of pH. Approximately 3.0 to 3.6 mg of alkalinity (as CaCO3) is produced per milligram of nitrate reduced to nitrogen gas. Optimum pH values for denitrification are between 7.0 to 8.5.

Chapter 6 - QA/QC (Quality Assurance/Quality Control)

Section 6.1 - Definitions

6.1.1 Define Quality Control.

Quality control (QC) is a technical, operational function which investigates and confirms the proper conduct of all those procedural components necessary to a successful conclusion.

Quality control is a variety of techniques that the sampler and analyst perform to verify that the sampling and analytical protocols meet the desired goals for data quality. QC functions help to ensure data validity and traceability.

An example of QC is the incorporation of QC samples such as blanks, replicates, and spikes into the analysis. The QC process ends at the assignment of QC sample frequencies and acceptance criteria. It is the Quality Assurance aspect that actually evaluates the effectiveness of a QC program.

6.1.2 Define Quality Assurance.

Quality assurance (QA) is described as a management function which rests on the documentation and establishment of quality control protocols, and on the evaluation and summarization of their outcomes.

Quality assurance is the system for checking and ensuring that quality control criteria are appropriate based on the desired level of quality (precision and accuracy) of data being generated. Quality assurance includes corrective action protocols.

The role of quality assurance in the laboratory is to ensure that the quality control program will guide the laboratory towards generating data which meets its goals for accuracy and precision. In the case of Acme Laboratory, this lab could actually forget to spike the

samples, obtain a recovery of 0%, and pass muster! The role of the quality assurance program then, is to continually review all aspects of the quality control program and make adjustments —or initiate corrective action— as needed to achieve the laboratory's data quality goals.

An example of QA in action is determining that matrix spike control limits of 0 to 200% are inappropriate for generating quality analytical results. A QA program should investigate reasons causing the excessively broad control limits and make adjustments to the QC program to improve the control limits.

6.1.3 Define Linear Regression.

Linear regression is a statistical tool for determining the relationship that exists between a dependent variable (instrument response, such as absorbance) and an independent variable (concentration), for a given set of data (calibration standards). As with other statistical tests, the more data provided, the more accurate the relationship will be defined. For instance, a linear regression based on seven (7) calibration standards spanning a concentration range of 0.1 to 1 ppm will be far more accurate than one based on only three (3) standards over the same concentration range.

As the name suggests, linear regression results in an equation for the straight line which describes the relationship. The important parameters that result from a regression are the slope and intercept of the resultant line. The correlation coefficient can also be calculated to provide an estimate of the strength or validity of the relationship between concentration and response.

The math behind the commonly used linear regression function is to adjust the values of slope and intercept to find the line that best predicts response (Y) from concentration (X). More precisely, the goal of regression is to minimize the sum of the SQUARES of the VERTICAL distances of the actual data points from the regression line; this is why it is commonly known as the "least squares" technique.

Understanding the rationale for minimizing the "sum of squares" is critical to understanding how linear regression works. If random data variability follows a normal distribution, it is far more likely to have two data points each with moderate deviations (say 5 units each) than to have one data point with a small deviation (1 unit) and one with a large deviation (9 units) from the regression line. A procedure that minimizes the sum of the absolute value of the distances would have no preference over a line that was 5 units away from two points and one that was 1 unit away from one point and 9 units from another. The sum of the distances (more precisely, the sum of the absolute value of the distances) is 10 units in each case. However, a procedure that minimizes the sum of the squares of the distances prefers to be 5 units away from EACH of two points (sum-of-squares = $5^2 + 5^2 = 25 + 25 = 50$) rather than 1 unit away from one point and 9 units away from another (sum-of-squares $1^2 + 9^2 = 1 + 81 = 82$). Assuming that random variability follows a normal distribution, the line determined by minimizing the sum-of-squares is most likely to be correct.

6.1.4 Define Correlation Coefficient.

Correlation addresses the relationship between two different factors (variables: X and Y). The statistic is called a correlation coefficient. A correlation coefficient can be calculated when there are two (or more) sets of scores for the same individuals or matched groups.

A correlation coefficient describes direction (positive or negative) and degree (strength) of relationship between two variables. The quantity r, called the correlation coefficient, measures the strength and the direction of a linear relationship between two variables. The higher the correlation coefficient, the stronger the relationship. See Figure 6.1.04 for an illustration of various data plots with associated correlation coefficients.

Examples:
Positive correlation: If X and Y have a strong positive linear correlation, r is close to +1. A r value of exactly +1 indicates a perfect positive fit. Positive values indicate a relationship between X and Y variables such that as values for X increase, values for Y also increase.

Negative correlation: If X and Y have a strong negative linear correlation, r is close to -1. A r value of exactly -1 indicates a perfect negative fit. Negative values indicate a relationship between X and Y such that as values for X increase, values for Y decrease.

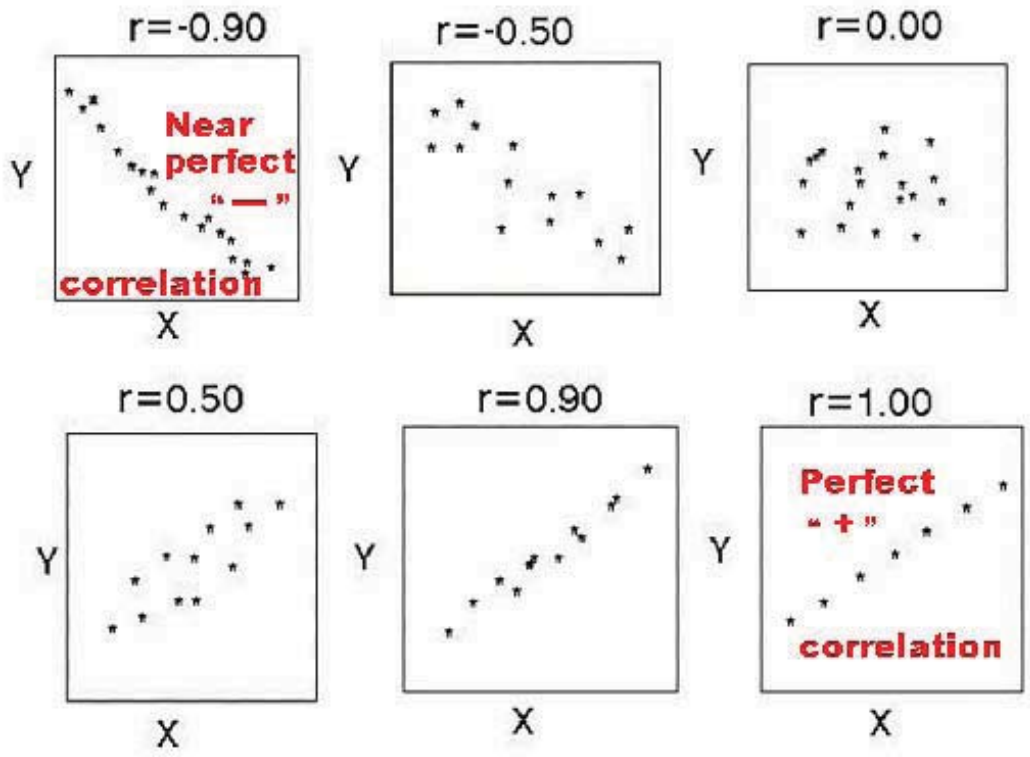
No correlation: If there is no linear correlation or a weak linear correlation, r is close to 0. A value near zero means that there is a random, nonlinear relationship between the two variables.

Note that r is a dimensionless quantity; that is; it does not depend on the units employed.

A perfect correlation of ± 1 occurs only when the data points all lie exactly on a straight line. If r = +1, the slope of this line is positive. If r = -1, the slope of this line is negative.

A correlation greater than 0.8 is generally described as strong, whereas a correlation less than 0.5 is generally described as weak. These values can vary based upon the "type" of data being examined. A study utilizing scientific data may require a stronger correlation than a study using social science data.

Figure 6.1.04



6.1.5 Explain the difference between Sample Standard Deviation and Population Standard Deviation.

The "population" standard deviation is the statistical term for the standard deviation of all possible values for a specific parameter. In the real world, finding the standard deviation of an entire population is unrealistic except in certain cases, such as standardized testing, where every member of a population is sampled. An example might be calculating the specific accuracy of every piece of volumetric glassware made in a certain lot. The "sample" standard deviation is what we commonly use to estimate the "population" standard deviation, because it's unrealistic to expect that we can make every measurement possible. Therefore, we perform a limited number of measurements and from those data values, we calculate the "sample" standard deviation, which we believe to be an estimate of the actual "population" standard deviation.

How good an estimate is the "sample" standard deviation of the "population" standard deviation? That depends on the sample size tested. If you only take three (3) measurements, your calculated "sample" standard deviation will likely be quite different than if you calculate it based on 20 measurements. Basically, the more values we use to calculate the "sample" standard deviation, the closer the result will be to the actual "population" standard deviation. Scientifically speaking, once you make at least 20 measurements, the statistical difference between the "sample" and "population" standard deviation becomes insignificant. Notably, that is why statistical control limits must be generated using at least 20 data points.

The difference in the calculation is in the formula, where the denominator used is (n-1) for the "sample" standard deviation while (n) is used for the "population" standard deviation. On some calculators, there is a button labeled as "d-1" or "n-1" for the "sample" standard deviation, and a button labeled as "d" or "n" for the "population" standard deviation.

The formula for calculating standard deviation and the differences between the two forms is outlined below:
1. For each individual data point x, subtract the overall mean of all data from the individual data point (x), then multiply that result by itself (otherwise known as determining the square of that value).
2. Sum up all those squared values.
3. Divide the result obtained in step 2 by the number of data points minus 1 (n-1).
4. The square root of the resulted obtained in step 3 represents the SAMPLE standard deviation of your set of data.
To obtain the POPULATION standard deviation, in step 3, divide by the total number of data points (n) instead of (n-1).

In Excel, the formulas for the two functions are as follows:

STDEV(range of cells) = sample standard deviation formula

STDEVP(range of cells) = population standard deviation formula
(STDEVP should never be used)

6.1.6 Define Outlier.

In statistics, an outlier is an observation that is numerically distant from the rest of the data. They can occur by chance in any distribution, but they are often indicative either of measurement error or that the population has a skewed distribution. In the former case one wishes to discard them, while in the latter case they indicate that one should be very cautious in using tools or intuitions that assume a normal distribution. A frequent cause of outliers is a mixture of two distributions (often called a bi-modal distribution), which may be two distinct sub-populations, or may indicate "correct trial" versus "measurement error". A good example of a bi-modal distribution of data could be seen in PT sample results. For example, with PT samples for cBOD, there are frequently a large number of results which are significantly higher than the majority of the data. An investigation would likely find that those labs that reported the high bias or “outlier” results likely forgot to add inhibitor to the samples. This is a clear case where the results may be outliers which can easily be explained based on analytical error. See Figure 6.1.06A for an illustration of outlier data points that don't fit the resultant regression line (or curve). See Figure 6.1.06B for an illustration of how outliers can affect control charts.

In most larger samplings of data, some data points will be further away from the sample mean than what is deemed reasonable. This can be due to incidental systemic error or flaws in the theory that generated an assumed family of probability distributions, or it can

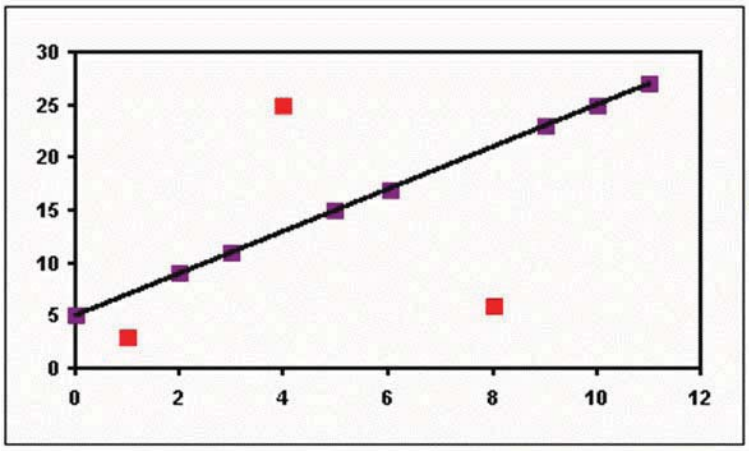
simply be the case that some observations happen to be a long way from the center of the data. Outlier points can therefore indicate faulty data, erroneous procedures, or areas where a certain theory might not be valid. However, a small number of outliers not due to any anomalous condition is to be expected in large samples.

Outliers, being the most extreme observations, will include the sample maximum or sample minimum, or both, depending on whether they are extremely high or low. However, the sample maximum and minimum need not be outliers, if they are not unusually far from other observations. Outliers should not routinely be removed from a set of data without justification. Statistically determined outliers should be plotted on control charts, but not used to calculate quality control limits.

In Figure 6.1.06A, the data points that do not fall along the trend lines, depicted in red, are outliers by this definition. There is still a problem with this definition for our purposes, and that is statistical significance.

In the graph on the upper left of Figure 6.1.06A, we see that three of eleven data points qualify as outliers. Any of you that have ever taken a statistics course will immediately agree that nearly 30% of all collected data points cannot be dismissed as outliers. So, how do we determine how many data points we can reasonably dismiss as outliers? To answer this question, we must first determine if the sample of measurements is statistically significant. You need to mathematically calculate statistical significance or reliability (i.e. Grubbs Test), based on sample size.

Figure 6.1.06A



■ Representative data points

■ Suspected outlier data points

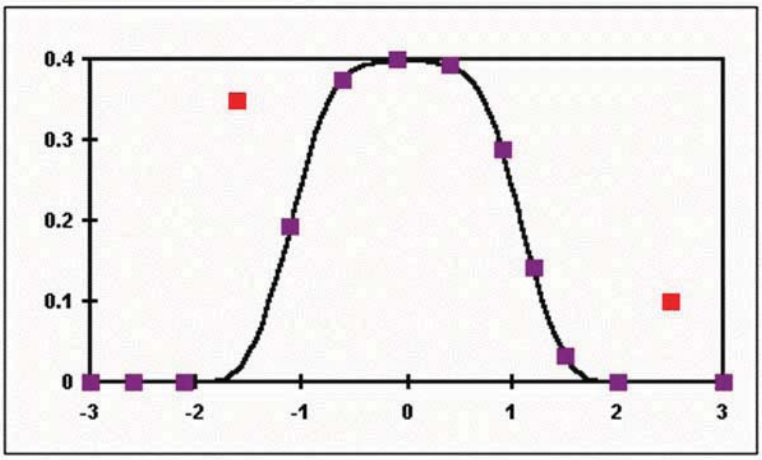
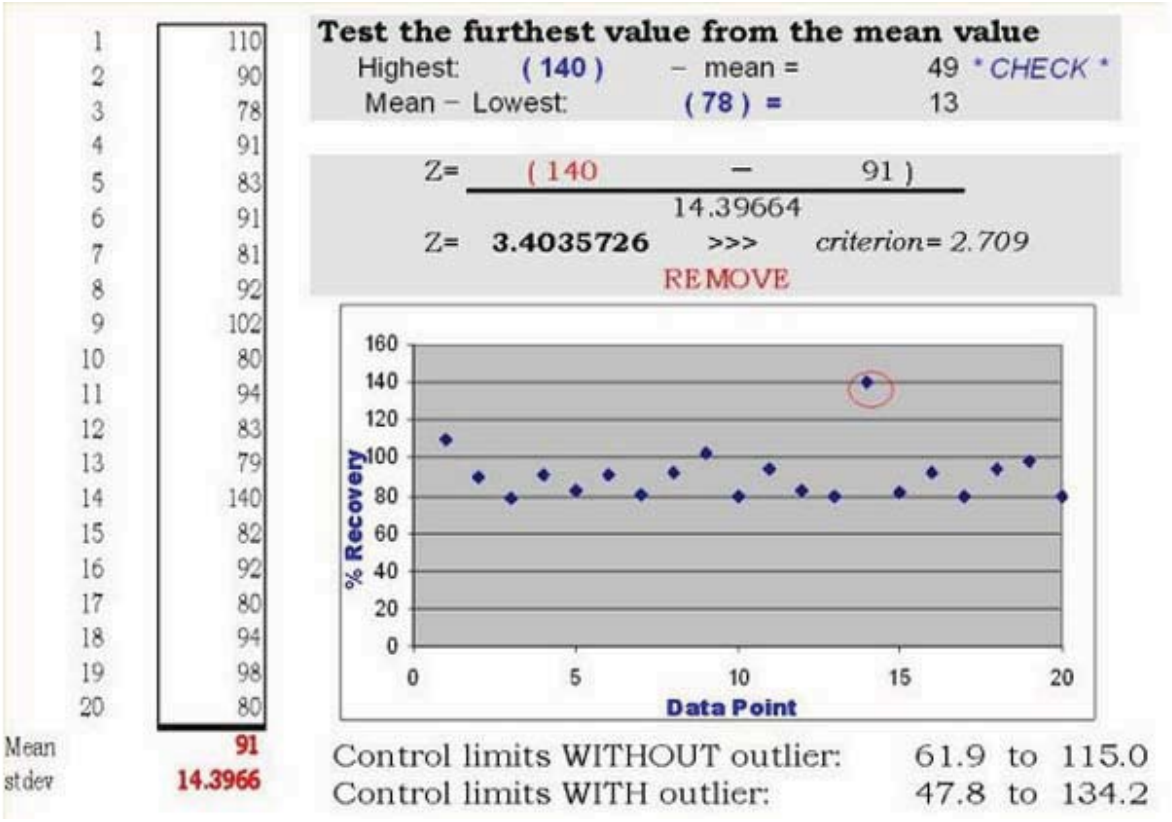


Figure 6.1.06B



Section 6.2 - Precision / Accuracy

6.2.1 What is the basic math behind calculating Standard Deviation.

The measure of the dispersion of a set of data from it's mean (average). The more spread out the data is, the higher the standard deviation. It is calculated like this:

x = one value in your set of data
avg (x) = the mean (average) of all values (x) in your set of data
n = the number of values (x) in your set of data

1. For each value (x), subtract the overall avg (x) from x, then multiply that result by itself (otherwise known as determining the square of that value).
2. Sum up all those squared values.
3. Then, divide THAT result by (n-1).
4. The squre root of the result from STEP 3 is the standard deviation.

To better understand the concept of standard deviation, we have to consider the normal distribution of data, often termed the "bell-shaped" curve.

In a normal distribution, the values falling within one standard deviation of the mean (red areas only), generally represent about 68% of all values. Two standard deviations away from the mean (the red and green areas) account for a little more than 95 percent of the

values. And three standard deviations (the red, green and blue areas) account for about 99.7 percent of the values.

Calculation of Standard Deviation the Hard Way

1. Find the sum of the total number of % recoveries (or whatever you are using for data).
2. Find the average percent recovery for those values by dividing the total by the number of values.
3. Subtract the average percent recovery from each value. Do not use - or +.
4. Square all of the numbers you got in step 3.
5. Total all the numbers you got from step 4.
6. Find the average of the total be dividing it by N-1 (i.e., if you had 20 values, you would divide by 19 (20-1)).
7. Find the square root of the number from step 6.

Example: Find the Standard Deviation of the following numbers.
216, 128, 77, 248, 147, 228, 164, 98, 114, 175, 205, 172, 210, 172, 117, 177, 187, 123, 210, 85

Step 1. Add all the values. Sum should be 3,253.

Step 2. Divide by the total values (20) to get the average. It should be 163.

Step 3. Subtract 163 from each value, then square each value.

Step 4. Total the squared numbers from 3. You should have 47,823.

Step 5. Divide this sum by 20-1 = 19. You should have 2,517.

Step 6. Find the square root of 2,517 It should be 50.17. This is the standard deviation of your set of % recoveries.

6.2.2 What is the basic math behind calibration curve generation?

Calibration Curve Generation
Linear regression is required for total phosphorus calculations. The algebraic formula for the equation of the best-fit line that fits a set of data is

$y = mx + b$

where:

y = response (absorbance) [as the dependent variable, this must be plotted on the y (or vertical) axis]

x = concentration (mg/L) [as the independent variable, this must be plotted on the x (or horizontal) axis]

m = slope

b = y-intercept

The slope and y-intercept are determined from a calibration curve that consists of at least

three different concentration standards and a calibration blank. The calibration blank, the zero concentration standard, is not automatically assigned a zero response. The calibration blank uses zero for the concentration and the measured absorbance for the response.

The linear regression formula is:

Y (absorbance) = M (slope) times X (concentration)
plus B (y-intercept)

Since this formula (as written above) "solves" for absorbance (which is the variable we already know) we need to rearrange the formula so that it "solves" for concentration which is the unknown variable in the samples.

Therefore to solve for X (concentration) we rearrange the formula to read:

$$X\,(\text{concentration}) = \frac{Y\,(\text{absorbance}) - B\,(\text{y-intercept})}{M\,(\text{slope})}$$

Be aware that different calculators and even MS Excel TM software can provide slope and intercept functions that are inverse regression coefficients from the standard [y = mx + b] equation. As such, when inverse regression coefficients are used, the equation changes to:

X (concentration) = M (slope) × Y (absorbance) + B (y-intercept).

Note that when using this equation, concentration is still the independent variable and response is the dependent variable.

The key is to be able to identify and understand which results your calculator or spreadsheet provides.

The easiest way to determine which equation to use is determine the "response factor" (RF) of your midpoint calibration standard. Take your midpoint standard and divide the concentration by its absorbance. If the result is greater than 1 (i.e., concentration is greater than absorbance) then the slope for the linear regression should be less than 1. This is because slope is mathematically defined as the "rise" (increase in absorbance) over "run" (increase in concentration). Looking at it logically, if the response factor is greater than 1, then using the standard equation [Y = mX + b] would require the slope to be greater than 1.

For phosphorus in most small WWTP labs, absorbance is typically about 0.3 to 0.5, meaning absorbance is about 1/3 to 1/2 of the actual sample concentration. Of course, this depends on the cell path length.

If the RF is > 1 and the slope (M) is < 1 or the RF is < 1 and the slope (M) is > 1 then calculate results as:

Concentration = absorbance × slope + intercept

If the RF is > 1 and the slope (M) is > 1 or the RF is < 1 and the slope (M) is < 1 then calculate results as:

Concentration = (absorbance - intercept) ÷ slope

6.2.3 Discuss why outlier rejection is important before calculating control limits.

First, it is critical to understand that the basic premise of any outlier test is simply to assign a statistical likelihood that a given suspect outlier point represents a different population than the other values in a data set. It cannot - and should not - tell you what to do with that point. Because the consequences associated with including outliers (control limits become unreasonably broad allowing inaccurate data) far outweigh the risk of excluding them (control limits become too restrictive and too many data points become suspect), we recommend that outliers be excluded from further use.

One has to remember the distinction between “out-of-control” and “outlier”. While outliers should certainly be excluded from calculation of new control limits, the term “outlier” is reserved for those data points which can be statistically defined as such. Simply put then, you MUST include all data points when calculating control limits unless:

(1) you have documentation which substantiates that an analytical error was made (e.g., you documented that you spilled some of the spike solution or spiked sample during preparation), or (2) you have performed a statistical test which has determined the value to be an outlier.

Outlier values act to increase the standard deviation of a set of data and also to increase (or sometimes decrease) the mean of a set of data. Since control limits are established by multiplying the mean of a set of data by either the standard deviation or a statistical factor designed to represent the standard deviation, any bias in the mean or standard deviation will result in an artificial broadening of the control limits. This can result in what maybe called the "Mack Truck" limits - anything will pass. Limits of this type have no value in monitoring the precision or accuracy of test results.

A simple example can be seen with a series of five (5) values: 95.2%, 97.6%, 101.3%, 102.7%, and 177.6%. If the preferred test to determine outliers, Grubb's test, is performed on this limited set of data, the value, "177.6" will be identified as an outlier. The mean of the data WITH the outlier is 114.9, yet the mean with the outlier excluded is 99.2, a significant reduction.

If one looks at the standard deviation, the standard deviation of the data WITH the outlier is 35.2, yet the standard deviation with the outlier excluded is only 3.43. The inclusion of the outlier increases the standard deviation nearly ten-fold.

If control limits were to be calculated based on this small set of data (mean ± 3 x standard deviation, the control limits WITH the suspected outlier included, the limits would be 9.3 to 220, or "mack truck" limits. If the outlier is excluded, however, the control limits become a

more reasonable 88.9 -110.

6.2.4 Discuss preparing matrix spikes - dilution to volume

Spikes can be prepared by either of two techniques: “dilution to volume” or “addition to volume”. See Figure 6.2.04A for a graphic illustrating the difference between the “Dilution to Volume” and “Addition to Volume” spike preparation techniques. With “dilution to volume”, the spike is added first and then sample (or reagent water for LCS) is used to dilute the volume to a known mark typically used for analysis. With “addition to volume”, the full sample volume as normally used is accurately measured out and then the spike solution is added as additional volume.

See Figure 6.2.04B for a graphic illustrating the correction to background sample concentration required when using the “Dilution to Volume” spike preparation technique.

DILUTION TO VOLUME

If you dilute even a small volume of spike solution (with sample) to a known volume, then the sample volume used for the spike is LESS than that in the unspiked sample. Subsequently, when calculating recovery, the actual sample concentration must be adjusted (lower) to account for the difference in volume. Since there is less sample volume in the spiked sample (than what is routinely used for analysis), then you just can’t simply subtract the concentration determined for the unspiked sample. Furthermore, because we’ve added volume from the spike solution, we have actually DILUTED the sample concentration.

Example:
1. 50 mLs of unspiked sample is measured to be 0.5 µg/mL (ppm) ammonia.
2. A spike is prepared by pipetting 10.0 mLs of a 1.0 µg/mL spike solution and then diluting to a volume of 50.0 mLs with sample.
3. The spiked sample result is 0.65 µg/mL (ppm).

Effectively this means there are actually only 40 mLs of sample used. Since only 80% (40/50) of the sample volume is used, then only 80% of the background sample concentration (0.8 x 0.5 µg/mL = 0.4 µg/mL) can be subtracted from the spiked sample result to account for the background.

The spike concentration is 0.2 µg/mL (10 µg divided by 50 mL). The spiked sample result was 0.65 µg/mL. We subtract 0.4 µg/mL to account for the background contribution of the sample itself. That leaves us with 0.25 µg/mL “recovered”.

Since we spiked 0.2 µg/mL, the spike recovery is 125% (0.25 µg/mL/0.2 µg/mL).

Figure 6.2.04A

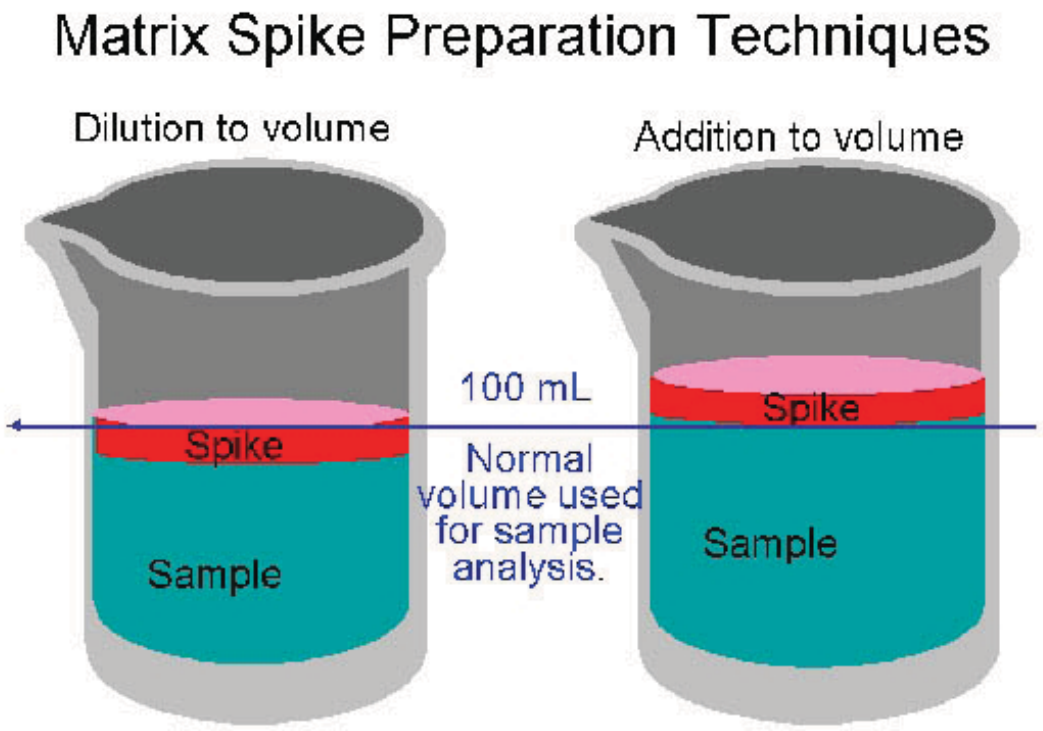
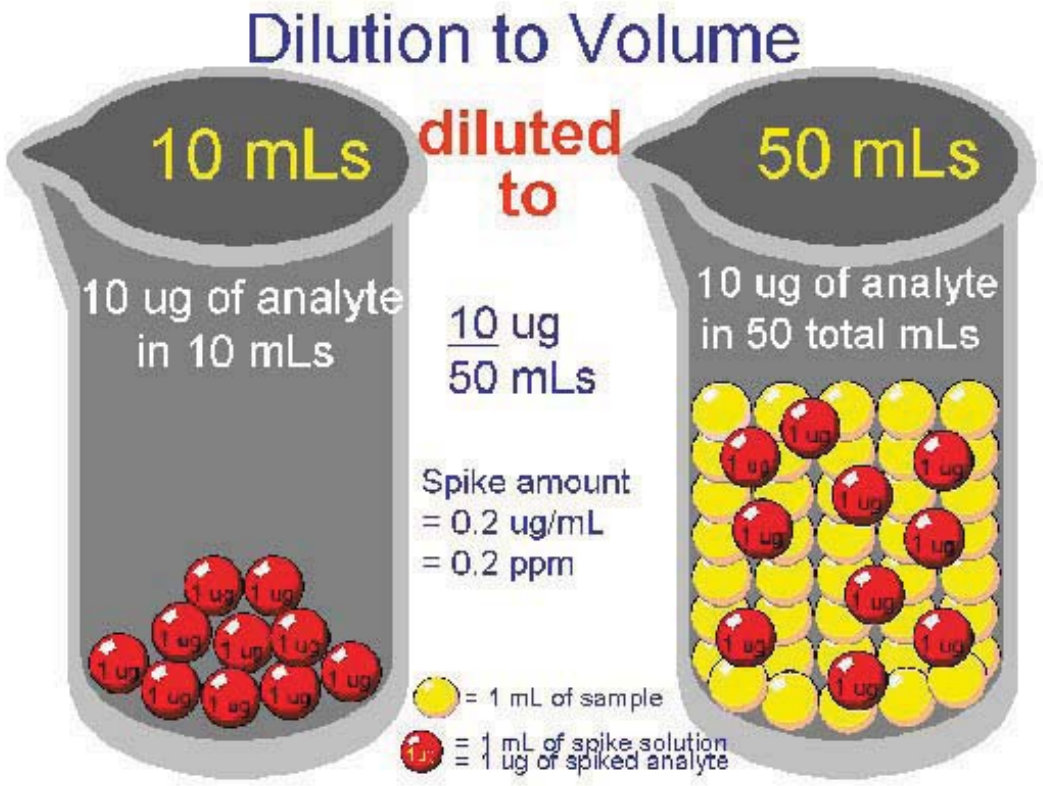


Figure 6.2.04B



6.2.5 Discuss preparing matrix spikes - addition to volume (adding on top)

If the spike is added "on top of" the sample, i.e., the amount of sample used in the spike is the same as in the unspiked sample, then the spike concentration must be adjusted. Analysts are often "tricked" into thinking that 10 mLs of spike solution added to 100 mLs of sample represents a 10-fold dilution of the spiking solution. The actual spike amount [concentration] is determined as the ratio of spike volume to the total sample volume multiplied by the spike solution concentration. See Figure 6.2.05A for a graphic illustrating the correction to spike concentration required when using the "Addition to Volume" spike preparation technique.

Addition to Volume - differential effects

If there is no pre-treatment that results in sample volume reduction involved (e.g., digestion, distillation), then TWO correction factors are required:
1. One for the dilution of sample concentration
2. Another for dilution of spike concentration

Example: Total phosphorus by autoclave.

If there is a pre-treatment step with sample volume reduction involved (e.g., digestion, distillation), then no correction factor is required. Examples: Total phosphorus by hot plate, distilled ammonia. See Figure 6.2.05B for a graphic illustrating why no correction is required for hotplate digestion of total phosphorus when using the "Addition to Volume" spike preparation technique.

Example:
1. 50 mLs of unspiked sample is measured to be 0.5 µg/mL (ppm) ammonia.
2. A spike is prepared by pipetting 10.0 mLs of a 1.0 µg/mL spike solution and then adding it to a volume of 50.0 mLs with sample.
3. The spiked sample result is 0.60 µg/mL (ppm).

The concentration of the ammonia in the spiked sample has been diluted by the additional volume added by the spike. Effectively this means the original concentration has been diluted by 20%. Because of the dilution (50/60) only 83.3% of the background sample concentration (50/60 = 0.833; 0.833 x 0.5 µg/mL = 0.416 µg/mL) can be subtracted from the spiked sample result to account for the background.

The spike concentration is 0.167 µg/mL (10 ug divided by 60 mL). The spiked sample result was 0.60 µg/mL. We subtract 0.417 µg/mL to account for the background contribution of the sample itself. That leaves us with 0.183 µg/mL "recovered". Since we spiked 0.167 µg/mL, the spike recovery is 109.6% (0.183 µg/mL/0.167 µg/mL).

Figure 6.2.05A

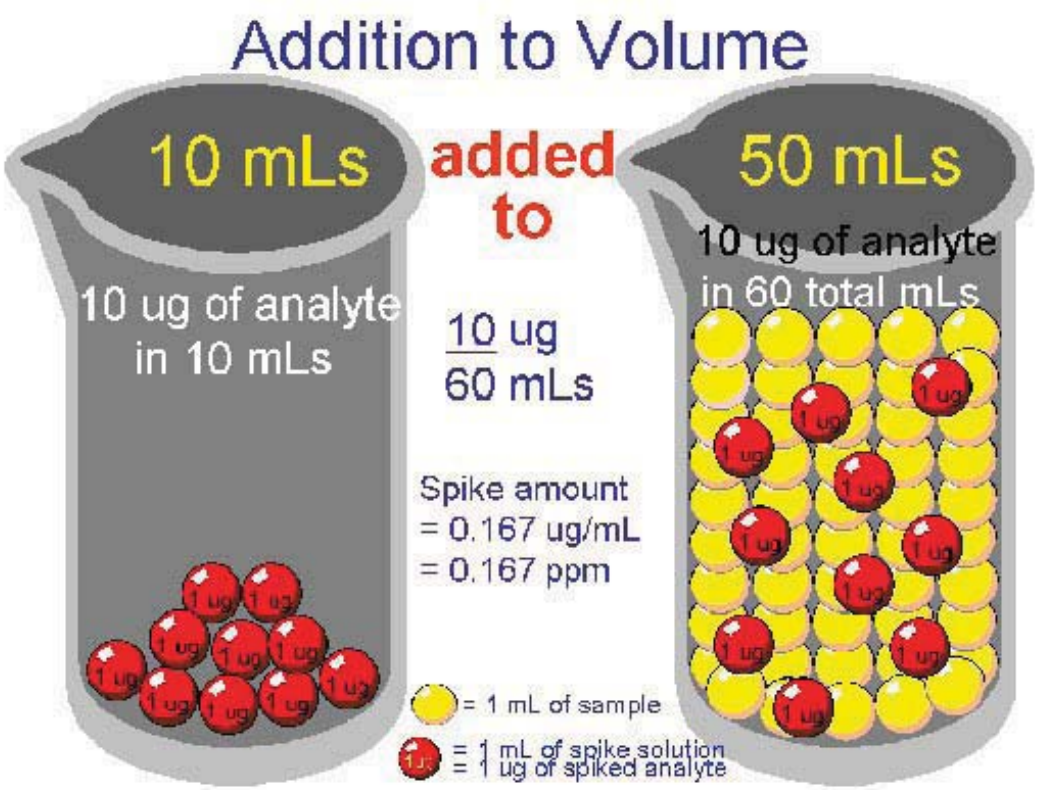
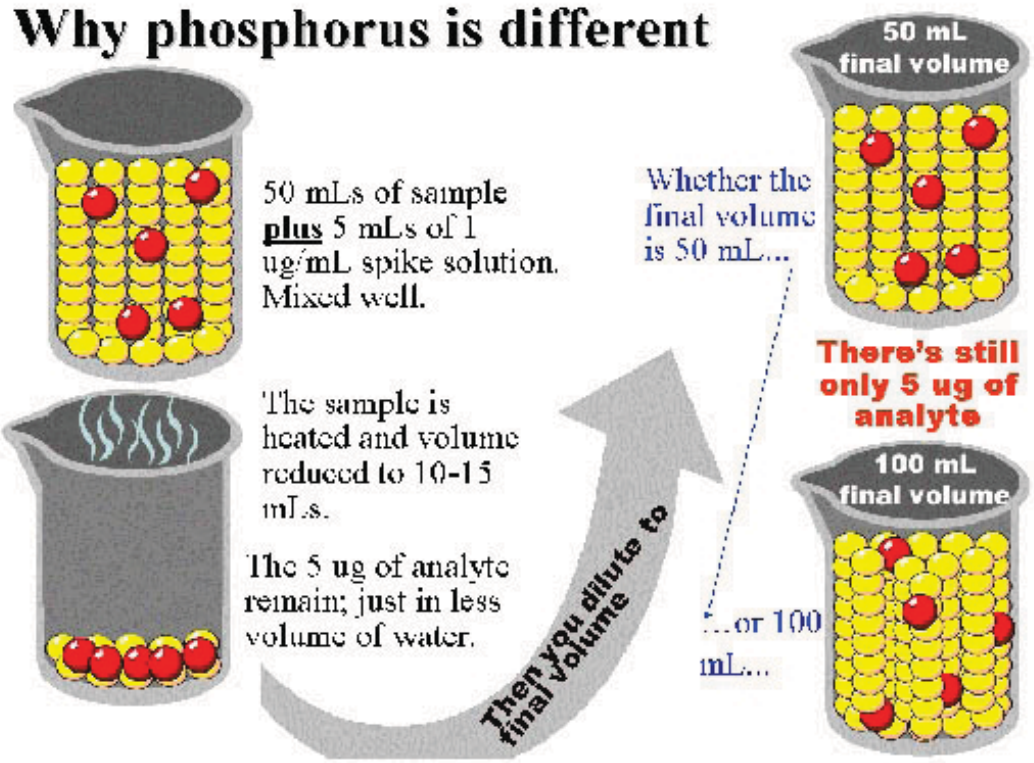


Figure 6.2.05B



6.2.6 Explain why QC results that exceed control limits should be included on control charts.

Occasionally, after calculating new control limits, the limits are perceived to be too restrictive for ordinary work. An example of this might be matrix spike control limits of 96 to 104%. Clearly, there is little room for error in these situations.

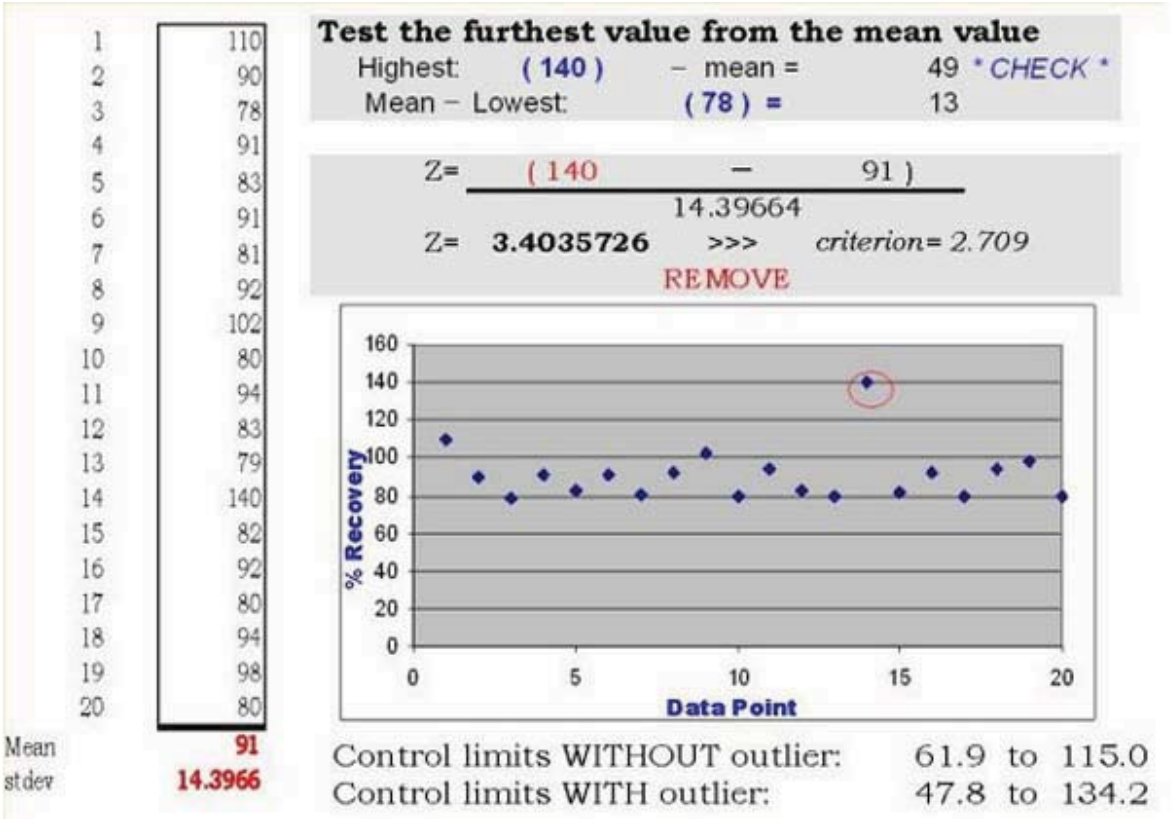
The statistical procedure used in the calculation of control limits is designed to produce limits at the 99% confidence level. In layman's terms, this means that only 1 out of every 100 data points is expected to be judged incorrectly (i.e., a point outside of control limits that should really be in-control, or a point within the control limits that should more appropriately be out-of-control). The range of values covered by the mean plus or minus 2 standard deviations is used to obtain warning limits. Statistically speaking, these are 95% confidence limits, which means that 5 out of 100 values (1 out of every 20) is expected to naturally occur outside of these boundaries.

What all of this means is that under normal circumstances, only 1 out of 100 data points is expected to fall outside of control limits—whether that be precision or accuracy. Many laboratories, however, might feel that "out-of-control" data points are found much more frequently. What typically is occurring in these cases however, is that either (1) control limits are not be calculated correctly, (2) there is some inherent bias in the procedure, or (3) some procedural error is at fault.

One of the most common causes behind QC sample failure is that control limits have become unreasonably restrictive. Invariably, this anomaly results from the exclusion of any out-of-control data point from use in constructing future control limits. The misconception is that because the data point is technically "out-of-control", it is not a valid data point, and thus should be excluded. In reality, it is precisely these occasional excursions that provide for a more realistic standard deviation, which in turn affects control limits.

It is important to remember that the quality control results used to generate a control chart are NOT plotted on that chart. Those limits should be used to construct a new, blank, QC chart-- which is filled in over time as future data are generated.

Figure 6.2.6.1



6.2.7 Discuss how to identify if results exceed the calibration range of an instrument and action to be taken.

Samples having RESPONSES (not concentration) greater than that of the most concentrated standard of an initial calibration, established using at least 3 different standard concentrations, must be diluted and reanalyzed.

When samples cannot be diluted and reanalyzed (i.e., beyond holding time, or insufficient sample remains), sample results shall be reported with appropriate qualifiers or narrative warnings.

It is critical to note that a calibration is established based upon absolute response as a function of concentration. Subsequently, the determination of whether or not a sample exceeds the calibration range is based on its absolute response rather than concentration.

What is the proper way of determining whether a sample requires dilution?

Given that:
SR = Sample Response (absorbance, etc.)
SC = Sample Concentration

UCSR= Upper Calibration Standard Response (absorbance, etc.)
UCSC= Upper Calibration Standard Concentration

The following sample would require dilution and reanalysis

SR= 0.915 | SC= 0.98 mg/L | UCSR=0.900 | UCSC= 1.00 mg/L

…because the sample response exceeds the response of the uppermost calibration standard. Even though the concentration determined by linear regression is less than that of the highest calibration standard, the sample must be diluted because we are really calibration response. Response is the KNOWN (independent variable). Sample concentration is the UNKNOWN.

The following sample would NOT require dilution and reanalysis:

SR= 0.875 | SC= 1.10 mg/L | UCSR=0.900 | UCSC= 1.00 mg/L

…because even though the sample concentration exceeds the calibration range (the concentration of the uppermost calibration standard), the sample response is well below that of the uppermost calibration standard.

6.2.8 Using the given data, calculate the concentration of spike added to a sample.

Given: Volume of Standard = 5.0 mL
Concentration of Standard = 100 mg/L
Volume of Sample = 100 mL

FORMULA:

$$\text{Spike Added (mg/L)} = \frac{\text{Standard Volume (mL)} \times \text{Conc. Of Standard}}{\text{Standard Volume (mL)} + \text{Sample Volume (mL)}}$$

$$\text{Spike Added (mg/L)} = \frac{5\ \text{mL} \times 100\ \text{mg/L}}{5\ \text{mL} + 100\ \text{mL}} = \frac{500}{105}$$

$$= 4.76\ \text{mg/L}$$

6.2.9 Calculate the percent recovery using the given spiked sample analysis.

Given:
Observed Concentration = 56.75 mg/L
Background Concentration = 50.0 mg/L
Spike Concentration = 7.80 mg/L

FORMULA:

$$\% \text{ Recovery} = \frac{(\text{Observed Conc.} - \text{Background Conc.})}{\text{Spiked Conc.}} \times 100$$

$$= \frac{56.75 - 50.0}{7.80} = \frac{6.75}{7.80} = 86.5\%$$

6.2.10 Discuss the types of QC samples that are used to assess accuracy and those used to assess precision.

QC samples used to assess accuracy:
Accuracy is a measure of the proximity of an unknown to the "true value" or the expected result.

PT (an external unknown standard; goal: determine true value)
QCS (an external known standard; goal: determine true value)
ICV (an internal known standard; goal: determine true value, validate calibration)
LCS (an internal known standard; goal: determine true value)
Matrix Spikes (an internal known addition; goal: recover true value)
Matrix Spike Duplicates [% Recovery] (an internal known addition; goal: recover true value)
Surrogates (an internal known addition; goal: recover true value)
Split samples [sent to a contract lab] (goal: determine which lab is correct)

QC samples used to assess precision:
Precision is a measure of the reproducibility of an analysis or the ability to obtain the same result on consecutive measurements of the same sample. In order to determine precision, then, multiple sample analyses are required.

Replicates [duplicates] (goal: reproducibility)
Matrix spike duplicates [range or RPD] (goal: reproducibility)

Section 6.3 - Limit of Detection (LOD)

6.3.1 Discuss what to do if your LOD seems unreasonable.

Prior to reporting a calculated LOD, the analyst should ask: Is this LOD reasonable and if not, what can be done to improve the determination? Analyst experience is an important factor when deciding whether or not a calculated LOD is valid and analytically achievable. It is often useful to run the LOD study at several concentration levels over a long period of time and compare the results. This allows the analyst to become familiar with how the system operates and what sensitivity can be expected at varying concentrations.

The easiest way to prove that your LOD is unreasonable is to prepare a standard equal (or very close to) the calculated LOD and analyze it as a sample. Did you detect it? Or was it barely distinguishable from a blank? If you can't accurately (within 30% of true value) quantitate a standard prepared at your LOD, the LOD is unreasonable.

Why does this happen? Some instruments are simply too precise. In this case you could try analyzing the LOD replicates over several days or alternatively, intersperse replicates with

real samples.

If all else fails, you can "back into" your realistic LOD:
1. Prepare and analyze a blank spike at a concentration equal to your calculated LOD/MDL.
2. Can you quantitate it within 20-30% of expected value? If not, repeat this process at a higher concentration until you achieve a quantitative result (within 20-30% of target value).

Example:
Analysis: Ammonia Spike Concentration: 0.5 mg/L
LOD (calc.): 0.009 mg/L

If you have repeated the "7 replicates" more than twice or you have reached the lowest recommended spike levels and you still don't have a valid LOD:
1. Prepare and analyze a single standard at (or close to) the calculated LOD.
[e.g., 0.1 mg/L].
2. If you obtain a result within 30% [0.007 - 0.013] of the concentration you prepared, then you have validated your LOD.
3. If you DO NOT obtain a value within 20-30% [suppose you get 0.001] of the prepared concentration, then...
4. Prepare another single standard at a concentration slightly higher
[e.g., 0.02 - 0.05 mg/L].
5. Repeat steps 2 - 4 until you are within 30% [0.014 - 0.026 mg/L if spike concentration = 0.02 mg/L].

Section 6.4 - Data Qualification

6.4.1 Discuss when it is appropriate to qualify data.

Essentially, data SHOULD be qualified whenever something that could affect the actual results, or interpretation of them, has occurred. Examples include, but are not limited to: unusual weather or sample conditions, exceedance of analytical holding times, sample preservation problems, samples were not collected or stored at the proper temperatures, quality control sample results associated with sample analysis failed to meet acceptance criteria.

NR 149, however, specifies that the lab must have and follow a written policy that clearly outlines the conditions under which samples will be accepted or rejected for analysis, or under which associated reported results will be qualified.

Qualified data does not mean bad data. It merely helps the user to interpret the data, particularly if unusual circumstances are involved. When making environmental decisions based on data, it is critical that ANY non-typical information about a particular data point be identified.

6.4.2 Discuss how to qualify data and when QC has been exceeded.

Qualify data or reject samples for analysis if:
• received beyond holding time
• improperly preserved

• received in inappropriate containers
• there is evidence that the samples were collected improperly
• there is insufficient volume to complete requested analyses at the required LOD

NR 149.47 (1)(e) 13: Unless otherwise specified by DNR, [or as exempted by (1)(c) and (d)], lab reports must include at least:
Any deviations from NR 149 or method requirements, when the deviations affect the validity/defensibility of results. These can be described by narratives, flags, or qualifiers. If use flags or qualifiers, a key to flag's meaning must be provided. If a lab reports any results from a subcontract lab, the lab must include any qualifiers reported by the subcontractor.

QUALIFYING DATA WHEN QC IS EXCEEDED

BLANKS [NR 149.48(3)(d)] Reanalyze (or qualify results of) any sample in a batch if the concentration batch method blank is greater than (the highest of):
• The limit of detection.
• Five percent of the regulatory limit for that analyte.
• Ten percent of the measured concentration in.

LCS [NR 149.48(4)(g)] If LCS does not meet acceptance criteria, the lab must reprocess & analyze or qualify results of all samples in the preparation batch.

MS/MSD [NR 149.48(6)(d)] If MS/MSD do not meet acceptance criteria, the lab must reprocess & analyze or qualify results of the spiked sample and any others in the preparation batch deemed to be affected.

REPLICATES [NR 149.48(7)(e)] If replicates do not meet acceptance criteria, the lab must reprocess & analyze or qualify results of that sample and any others in the preparation batch deemed to be affected.

6.4.3 Discuss how data is qualified on a DMR.

There are three key places on a DMR that are used to qualify wastewater data: the QC Exceedance box, the General Remarks box, and the Laboratory QC Comments Box.

1. QC EXCEEDANCE Box (under each parameter on the DMR)
Place an "X" in the "QC Exceedance" box if ANY QC Exceedance was observed for that parameter during the month reported.

2. GENERAL REMARKS Box
This box is reserved for comments OTHER than those related to laboratory QA/QC problems. Examples include:
- Sloughing of solids causing high values.
- Inability to obtain a sample.
- Autosampler temperature problems.
- Flooding related problems (if you're under water, you surely are not testing).

3. LABORATORY QC COMMENTS Box
This box is reserved for comments SPECIFICALLY related to laboratory QA/QC problems. As briefly as possible, identify what QC parameters were exceeded. Try to be as specific as possible. For example, if a QC sample exceeded the upper control limit, it is helpful to identify what the QC result was as well as what the control limit was. This helps the user to evaluate the relative impact of the failure on the quality of data associated with it.

Qualified data are NOT necessarily "bad" data.
ALL this means is that the data user (i.e., the DNR) needs to take into consideration the nature of the situation surrounding the qualification when interpreting the results.

- Make sure your comments are meaningful and understandable to the end user.
- Provide enough information so that the DNR can assess the data quality.
- Remember to include qualifiers from subcontract labs.
- Attach comments on a separate sheet if necessary. Write "see attachment" in the QC Comments box.

QC Examples - Blanks
Situation: Your BOD blank depletions have been unacceptable for the past week. You traced the problem to a new bottle of "Cowboy Bob's" distilled water.
- Qualifier of little benefit: "BOD blank failed".
- Very helpful qualifier: "11/10/08 to 11/17/01 - BOD blank depleted more than is allowed (0.2 mg/L). Blank depletions ranged 0.6 to 1.1 mg/L. Traced to new bottle of water."

QC Examples - LCS
Situation: Your BOD glucose-glutamic acid (GGA) exceeded acceptance criteria. You used a new lot of GGA standard the next day and results were fine.
- Qualifier of little benefit: "GGA exceeded acceptance criteria".
- Very helpful qualifier: "11/7/08 - GGA analyzed this day (235 mg/L) exceeded criteria (198 + 30.5). Repeated GGA with new lot on 11/12/08. Result was 202 mg/L".

Chapter 7 - Documentation and Traceability

Section 7.1 - Traceability

7.1.1 Discuss mechanisms that can be used to ensure that "electronic" records are both permanent and unalterable.

Many labs are routinely using computers to create and store their data. Just as pencil can be erased, so can a value in a spreadsheet be easily deleted with a single keystroke. Security of electronic records begins with the use of password-based computer systems. Access to computer applications used to record and store laboratory records must be controlled through the use of unique user IDs and passwords. In addition, systems must be designed to automatically log a user off after a certain period of inactivity at the keyboard (or mouse).

All electronic data must be regularly backed up onto media which will survive record

retention requirements. This may mean changing media used to back-up data as technology advances.

The single most important security measure for electronic records is using a system or application which includes an “audit trail”. An audit trail can be defined as "a record showing who has accessed a computer system and what operations he or she has performed during a given period of time." Specifically, if an electronic value for a sample result is changed, the audit trail function must record the date and time of the change, the user ID of the individual making the change, and must record the value that was changed. Many audit trail applications also require the user making a change to add a brief notation citing the reason the change is being made.

References and Resources

1. **EXAMPLE SMALL WASTEWATER TREATMENT PLANT LABORATORY QUALITY MANUAL**

   2008. 4th Edition. Department of Natural Resources, PO Box 7921, Madison, WI 53707.

   http://www.dnr.state.wi.us/org/es/science/lc/OUTREACH/6QM/A_QualMan_rev0.pdf

2. **WISCONSIN ADMINISTRATIVE CODE, NR 149: LABORATORY CERTIFICATION & REGISTRATION**

   Wisconsin Legislative Reference Bureau, One E Main St, Suite 200, Madison, WI 53701-2037 Reference Desk: 608-266-0341

   http://legis.wisconsin.gov/rsb/code.htm

3. **STANDARD METHODS FOR THE EXAMINATION OF WATER AND WASTEWATER**

   1992, 1995 and 1998. 18th, 19th, and 20th editions, American Public Health Association, Washington DC.

   http://www.apha.org

4. **WISCONSIN ADMINISTRATIVE CODE NR 219: ANALYTICAL TEST METHODS & PROCEDURES**

   Wisconsin Legislative Reference Bureau, One E Main St, Suite 200, Madison, WI 53701-2037 Reference Desk: 608-266-0341

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5. **Methods for Chemical Analysis of Water and Wastes**

   1983. EPA-600/-4-79/020, Environmental Monitoring and Support Laboratory, Cincinnati, OH

   http://www.epa.gov

6. **A User's Guide to Laboratory Services**

   1989. Arneson, Ronald. Wisconsin Department of Natural Resources, Office of Technical Services, P.O. Box 7921, Madison , WI 53707

   http://dnr.wi.gov/org/es/science/lc/PW/Contract.htm

7. **Controlling Wastewater Treatment Processes**

   1984. Cortinovis, Dan. Ridgeline Press, 1136 Orchard Rd, Lafayette, CA 94549

8. **Handbook for Sampling and Sample Preservation of Water and Wastewater**

   1982. EPA-600/4-82-029. US Environmental Protection Agency, Environmental Monitoring and Support Laboratory, Cincinnati, OH 45268

   http://www.epa.gov

9. **Laboratory Safety Manual**

   1969. Mallinckrodt Chemical Works, Science Products Division, St. Louis, MO 63160

10. Operation of Municipal Wastewater Treatment Plants

1990. Manual of Practice No.11 (MOP 11), 2nd Edition, Volumes I, II, and III. Water Environment Federation (Old WPCF), 601 Wythe Street, Alexandria, VA 22314-1994. Phone (800) 666-0206

http://www.wef.org

11. Operation of Wastewater Treatment Plants

1990. 3rd Edition , Volumes 1 and 2, Kenneth D. Kerri, California State University, 6000 J Street, Sacramento, CA 95819-6025. Phone (916) 278-6142

http://www.owp.csus.edu/training/

12. Simplified Laboratory Procedures for Wastewater Examination

1985. Water Environment Federation (Old WPCF), 601 Wythe Street, Alexandria, VA 22314-1994, Phone (800) 666-0206

http://www.wef.org

13. Wisconsin Administrative Code, NR 218, Method and Manner of Sampling

Wisconsin Legislative Reference Bureau, One E Main St, Suite 200, Madison, WI 53701-2037 Reference Desk: 608-266-0341

http://legis.wisconsin.gov/rsb/code.htm